GAS PHASE ORGANOPHOSPHATE DETECTION USING ENZYMES ENCAPSULATED WITHIN PEPTIDE NANOTUBES

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Abstract

Previous work to develop biosensors that can be used to detect organophosphorus compounds (OPCs) has successfully demonstrated the potential application of enzymes encapsulated in peptide-nanotubes (PNTs) enhanced with horseradish peroxidase (HRP) to detect the presence of OPCs in the aqueous and gas phases (Stevens, 2012; Park et al., 2011a; 2012b; Baker, 2013). This previous research demonstrated that PNTs provide more surface area for the enzyme-catalyzed chemical reaction; while HRP provides increased electrochemical sensitivity. In this research, a standardized test method developed by Baker (2013), which was applied to evaluate a biosensor fabricated with a single-use electrode, was refined to accommodate a reusable screen printed electrode. Also in this study, butyrylcholinesterase (BChE) enzyme was used in lieu of the acetylcholinesterase (AChE) enzyme applied in Baker's (2013) study in an effort to enhance biosensor performance.

Biosensor operation is based on the principle that butyrylthiocholine (BSCh), in the presence of the enzyme BChE, will produce a measurable electrochemical signal during chemical reaction; a signal that is inhibited in the presence of an OPC. For this research, cyclic voltammograms (CVs) were used to measure the inhibition in current at a specified voltage due to the presence of a model OPC, malathion. Inhibition of the signal produced by an AChE-based biosensor due to the presence of malathion was found to be proportional to the malathion concentration (Baker, 2013). In the current study, the response of a BChE-based biosensor was also shown to be inhibited by gas phase malathion concentrations less than 25 ppbv, with the extent of inhibition linearly

proportional to the malathion concentration above 6 ppbv. Additionally, this study demonstrated that a BChE-based biosensor stored at room temperature can be used as long as 42 days after fabrication.

To my wife, the center of my Universe.

Acknowledgments

My appreciation goes to the generous support provided by Ms. Dolores E. Ross at the Air Force Medical Support Agency (AFMSA) Office of the Surgeon General for Research and Development (SG5I) for her oversight, direction, and resources to address critical long-term challenges in support of the United States Air Force. I am also very thankful for my thesis advisor, Dr. Mark N. Goltz, for his time and encouragement as he guided my research effort and illuminated an infrequently traveled path for those bold enough to explore unresolved, critical issues questing for answers. Without his guidance, I'd have spent more time looking for a proverbial peptide-nanotube bioelectrical power switch instead of purposeful prospecting for answers. Also, my sincere gratitude to previous explorers for "ground breaking" research into aqueous and gaseous detection methods. My appreciation extends to Dr. Dong-Shik Kim and Peter Baker for their demonstration of potential application of enzymes encapsulated in PNTs to detect the presence of organophosphorus compounds (OPCs). I also very much appreciate both Dr. Sushil R. Kanel's and Dr. Daniel Felker's expertise, which was vital to my research guidance and direction. To the AFIT librarians whose special effort and assistance provided me with a wealth of information to conduct my research. Lastly, to everyone in my life who's provided opportunity, encouraged, or simply been there to share in the growth experience. May life always be about the journey, not the destination!

Christopher W. Edwards

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GAS PHASE ORGANOPHOSPHATE DETECTION USING ENZYMES ENCAPSULATED WITHIN PEPTIDE NANOTUBES

I. Introduction

Background

Organophosphorus compounds (OPCs), among the most toxic substances known, are used as chemical warfare agents (CWAs), agricultural pesticides, and insecticides. For example, dimethyl methyl phosphonate (DMMP), an OPC, is used as a flame retardant and can result in non-lethal, deleterious effects such as nausea and/or vomiting after only a ten minute exposure to air concentrations as low as 0.005-0.01 mg/m^3 (Goltz et al., 2011). Because OPCs may cause harm at very low concentrations, sensitive, fast and accurate sensors are necessary to protect those potentially exposed.

Current analytical techniques, such as gas and liquid chromatography, although very sensitive and reliable, have disadvantages. The US Air Force currently uses a field portable gas chromatograph/mass spectrometer (GC/MS), Hazardous Air Pollutants on Site (HAPSITE), which is heavy, expensive, needs specially trained personnel to operate, and requires up to thirty minutes per single measurement (Goltz et al., 2011). Long measurement times directly impact the ability to maintain mission readiness when responding to a chemical agent attack. The Air Force standard for mission capability restoration is resumption of the primary mission within two hours of a chemical attack (USAF, 2003; USAF, 2011). Clearly, if a chemical agent attack is suspected, there is a need to reduce the time between sampling and acquisition of actionable results (Goltz et al., 2011).

Time-consuming, expensive techniques performed by highly trained technicians are not suitable for most situations requiring immediate attention (Goltz et al., 2011). Disadvantages of current techniques have motivated investigators to search for more useful detection technologies (Liu and Lin, 2006). The application of electrochemical biosensors for chemical agent detection is one promising avenue for development because these sensors are relatively simple to make and can be tailored to suit specific requirements (Upadhyayula, 2012). Advancements in the nanotechnology field have resulted in the development of biosensors that are fabricated with peptide nanotubes (PNTs) to improve sensor performance (Berger, 2008). Previously, a biosensor for the detection of OPCs in the aqueous phase was successfully demonstrated by encapsulating acetyl cholinesterase (AChE) enzyme on peptide nanotubes (PNTs) (Stevens, 2012). Research at the Air Force Institute of Technology (AFIT) (Baker, 2013) demonstrated the feasibility of reliable OPC detection in the gas phase at malathion concentrations as low as 12.5 ppbv utilizing horseradish peroxidase (HRP) encapsulated within PNTs, with the acetyl cholinesterase enzyme (AChE) on the outside of the PNT, on a single-use, gold screen-printed electrode (SPE) using Nafion as a protective layer (Figure 1). Additional studies have shown that direct sensing of target chemicals can be accomplished by using highly sensitive biosensors (e.g., enzymes) with a strong affinity toward these target molecules (Arduini et al., 2007; Stevens, 2012; Park et al., 2011b; Baker, 2013; Upadhyay & Verma, 2013).

Biosensor Construction and Operation

Biosensors are a relatively new, inexpensive technology that can be used *in situ* to provide real time data. While living systems control cellular function through an array of enzymes, biosensors can be constructed using a single enzyme as a highly selective

sensing agent. Enzymatic reactions may be either reversible or irreversible (Arduini, 2012a). Also, although enzymes preferentially interact with their complementary compounds, they are vulnerable to degradation over time once fabricated and environmentally exposed. Temperature, pH, and humidity may impact enzyme stability (Stevens, 2012; Baker, 2013). Enzyme activity may be maintained by protecting the enzymes under Nafion or cellulose acetate layers and by storing in a properly controlled environment (Baker, 2013; Arduini et al., 2012a).

In a recent review of OPC biosensor studies (Arduini et al., 2010), it was found that about ten percent of the studies involved testing sensors with a specific commercial application in mind; two papers explored OPC detection in the gas phase. One set of experiments tested a Prussian blue silver screen printed electrode (SPE) using butyrylcholinesterase (BChE) as an enzyme and butyrylthiocholine (BSCh) as the substrate (Arduini et al., 2007). In that study, cellulose acetate was utilized to preserve enzyme activity. Inhibition of the BChE/BSCh reaction in the presence of Sarin (GB) gas was measured to indicate the GB presence (Arduini et al., 2007). Since 2010, a number of studies have investigated gas phase detection of OPCs using sensors that incorporate nanomaterials. Biosensor fabrication using nanomaterials allows for miniaturization, while maintaining sensitivity and decreased response time when compared to the HAPSITE GC/MS detection process (Alonso et al., 2011; Arduini and Palleschi, 2012b; Arduini et al., 2012a; 2013; Baker, 2013; Chen et al., 2012; Ju et al., 2011; Wang et al., 2011). Previous studies have successfully tested OPC detection one day after sensor fabrication, Arduini et al., 2007; 2010; Andreescu and Marty, 2006, and others have demonstrated sensor longevity of fifty days using vacuum seal techniques (Andreescu and Marty, 2006). While Baker (2013) demonstrated successful single use

AChE biosensors, comparison of longevity under "normal" environmental laboratory conditions or electrode reusability were not considered. This research explores the use of reusable SPEs in the fabrication of a BChE/BSCh biosensor using PNT nanomaterials to increase surface area/sensitivity while using HRP to lower electrical resistance and Nafion to extend longevity. This particular fabrication and research approach is new and provides for baseline comparison.

When the OPC malathion interacts with acetylcholinesterase (AChE), the chemical bonding process is irreversible (Arduini, 2012a). Unlike AChE which resides between nerve cells and facilitates intercellular electrical impulses, butyrylcholinesterase (BChE) is created in the liver and circulates within the bloodstream (Evtugyn et al., 2013). BChE has a high affinity for OPCs such as malathion (Arduini et al., 2012a). Baker's (2013) research investigated gas phase detection of OPCs and used AChE as the enzyme (Figure 2). As shown in Figure 2, AChE catalyzes the hydrolysis of acetylcholine (ACh) to produce acetic acid and choline. If an OPC is present, the hydrolysis reaction is slowed due to permanent chemical bonding of the OPC onto the AChE active site. Cyclic voltammeter (CV) measured the current response of the ACh hydrolysis reaction, as well as the extent of inhibition of the reaction due to OPC presence. Thus, a biosensor based on the AChE/ACh hydrolysis reaction can be used to determine the presence of an OPC.

BChE facilitates hydrolysis of butyrylthiocholine (BSCh) into butyrylic acid and thiocholine, much as AChE facilitates hydrolysis of ACh (Figure 3). Baker (2013), who used acetylthiocholine (ASCh) in place of ACh as the substrate, demonstrated that inhibition of the current response of the AChE/ASCh reaction by a gas phase OPC, as measured by a CV, was proportional to the OPC concentration. Like the AChE/ACh chemical reaction previously described, inhibition of the BChE/BSCh reaction in the

presence of an OPC can be directly measured with a cycle voltammeter (CV). BChE transports OPCs from the pulmonary system to other locations within the body, such as to nerve cells where AChE is present (Evtugyn et al., 2013). BChE demonstrates a different affinity to OPCs than AChE (Arduini et al., 2007; Arduini and Amine, 2014; Evtugyn et al., 2012).

As part of a Cooperative Research and Development Agreement (CRADA) with the University of Toledo (UT) and Kwangwoon University, AFIT has been involved in the development of biosensors based on catalytic reaction and biomaterials (Baker, 2013; Stevens, 2012; Park, 2011; Park and Kim, 2012a; Park et al., 2010; 2011a; 2011b; 2012b). In the current study, Baker's (2013) approach is closely followed. However, BChE and BSCh were used instead of AChE and ASCh as the enzyme and substrate, respectively. While Arduini's earlier publications (2007 – 2013) and successes with BChE motivated this research, continued biosensor development utilizing BChE in lieu of AChE appears warranted to exploit BChE's different chemical properties (Arduini & Amine, 2014). In this research, a reusable SPE, see Figure 4, measured the electro-chemical reactions depicted in Figure 3 (Andreescu et al., 2002; Andreescu and Marty, 2006). When the electro-chemical reaction in Figure 3 is exposed to OPCs, the reaction in step one is inhibited due to the lack of available BChE. The intermediate breakdown of choline in step two produces hydrogen peroxide (Andreescu et al., 2006). Hydrogen peroxide, through hydrolysis with HRP catalyst, is broken down into water. HRP facilitates electron flow, allowing the reaction to take place at lower voltage (Park et al., 2012b; Baker, 2013). This lowers the electrical resistance between the anode and cathode and increases BChE/BSCh reaction rate sensitivity.

To protect enzyme function in biosensors, peptide nanotubes (PNTs) have been used (Park et al., 2010; 2012a; 2012b). Protecting enzyme activity and stability increases shelf-life and performance of the biosensor. As depicted in Figure 4, biosensor fabrication may include application of a final top layer component. The top layer provides protection as well as assists with maintaining adhesion of the PNTs to the SPE. Nafion, a stable biocompatible Teflon based polymer, has been used to bind PNTs (and their associated enzymes) to an electrode (Baker, 2013). When used in combination, PNTs, HRP, and Nafion are used to: increase the contact area between the enzymes and chemical compounds, protect the enzymes, and increase sensor sensitivity.

Problem Statement

Because OPCs may cause harm at very low concentrations, sensitive and accurate sensors are necessary to protect military personnel and civilians (Goltz et al., 2011). To meet demand for lower fiscal resource consumption, smaller, less expensive detectors need to be developed. Such detectors could be worn by military service members, homeland security personnel, and industrial workers (e.g., civil engineering pest control personnel and chemical plant operators). In addition, these small and sensitive biosensors can be integrated into a remote detection array or used on unmanned aerial vehicles to protect a central unit, building complex, or large population centers from distant, detectable threats.

Two critical problems arise with enzyme-based biosensors: 1) enzyme deactivation over time, and 2) inadequate sensitivity to the target compound. The UT and AFIT researchers are addressing these problems by 1) using Nafion and PNTs to protect the enzymes, thereby increasing the biosensor's longevity, 2) using PNTs to facilitate contact between the enzymes and target compounds, and 3) using HRP to

facilitate electron flow, thereby enhancing sensitivity (Stevens, 2012; Park et al., 2012b; Baker, 2013; Upadhyay and Verma, 2013).

The objective of this research was to evaluate gas phase OPC biosensor detection based on electrochemical inhibition measurement of BChE facilitated BSCh hydrolysis. Section II is written in the Scholarly Article format. Appendix A provides summary sensitivity and longevity information with an outline of methods used. Appendices B and C provide chronological detail on the research experiments. Appendix D provides the chemical materials ordering list. This research explored small, reusable, and affordable BChE-based biosensors with low OPC gas concentration detection capability.

Scope and Approach

1. Encapsulate HRP in peptide nanotubes (PNTs) to effectively immobilize and protect the hydrolysis catalyst, then add butyrylcholinesterase (BChE) enzyme to the outside of the PNTs. Finally, use Nafion to adhere the PNT/enzyme combination to a reusable gold screen printed electrode (see Figure 5).

2. Use a cyclic voltammeter (CV) to obtain data that quantifies the inhibition response of the BChE/BSCh hydrolysis reaction to varying concentrations of a model OPC (e.g., malathion). Determine:

a. Sensitivity: determine the range over which the inhibition of the hydrolysis reaction is proportional to the concentration of the model OPC. In addition, determine the detection limit for malathion.

b. Longevity: for a given malathion concentration, determine how the inhibition response of the hydrolysis reaction to the presence of malathion is affected as a function of the time after sensor fabrication, when the sensor is stored at room conditions.

c. Reusability: assess the ability to utilize an SPE multiple times after an initial use, subsequent cleaning, and reapplication of new biosensor components to the SPE.

II. Scholarly Article

Peptide Nanotube Encapsulated Enzyme for Vapor Phase Detection of Organophosphorus Compounds

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Abstract

Previous studies demonstrated the potential application of a biosensor fabricated with an acetylcholinesterase (AChE) enzyme encapsulated in peptide-nanotubes (PNTs) and enhanced with horseradish peroxidase (HRP) to detect the presence of organophosphorus compounds (OPCs) in water and gas phases (Stevens, 2012; Baker, 2013). The biosensor was fabricated using a single-use screen printed electrode (SPE). In the current study, potential improvements to the biosensor are investigated. The current study explores use of a butyrylcholinesterase (BChE) based biosensor using reusable SPEs that have a smaller working surface area than the single-use electrodes studied previously.

BChE-based biosensors were fabricated using PNTs, HRP, and Nafion in combination to increase reactive surface area, enhance sensitivity, and maintain enzyme stability. Cyclic voltammeter (CV) was used to measure gas phase concentration of the OPC malathion. Results of this research showed that a BChE-based biosensor could reliably measure gas phase malathion concentrations between 6 and 25 ppbv by current inhibition, with the extent of inhibition linearly proportional to the malathion concentration. The biosensors could be stored several weeks after fabrication at room temperature with minimal performance degradation. The electrodes were each reused

several times, and still were useable at the conclusion of this study. This research demonstrates the potential of fabricating a reusable, inexpensive biosensor capable of OPC detection with sensitivity and detection limit comparable to biosensors fabricated in previous studies.

Introduction

Organophosphorus compounds (OPCs) include insecticides and warfare agents which irreversibly bind to acetylcholine esterase (AChE) receptors in the central nervous system. OPCs prevent the nervous system from hydrolyzing acetylcholine (ACh), which consequently builds up. ACh stimulates muscles, and when an OPC has irreversibly bound to AChE, ACh accumulates, resulting in continuous stimulation of muscle groups. When a vital muscle such as the diaphragm cannot relax, suffocation results and causes death within minutes (Boss et al., 2010). Based upon the interaction of OPCs with AChE, previous studies have investigated use of esterase-based biosensors to detect OPCs in liquid (Park et al., 2011; Stevens, 2012) and gas (Baker, 2013) phases. Detection is based upon a redox reaction facilitated by the presence of AChE, using ACh or ASCh (acetylthiocholine) as substrates. OPCs inhibit the reaction, and the concentration of the OPC can be determined by using a cyclic voltammeter to measure the extent of inhibition. The AChE-based biosensor is fabricated on a gold screen printed electrode (SPE) using peptide nanotubes (PNTs), Nafion, and horseradish-peroxidase (HRP) in combination (see Figure 1) to increase reactive surface area, enhance sensitivity, and preserve enzyme stability over time (Arduini et al., 2007; 2013). Baker (2013), who used acetylthiocholine (ASCh) as the substrate, demonstrated that inhibition of the current response of the AChE/ASCh reaction (see Figure 2) by a gas phase OPC was proportional to the OPC concentration. Baker's work was carried out on single use, disposable SPEs and closely paralleled a similar research effort by Arduini (2012b) who also developed a disposable electrochemical biosensor with a shelf-life of about fifty days.

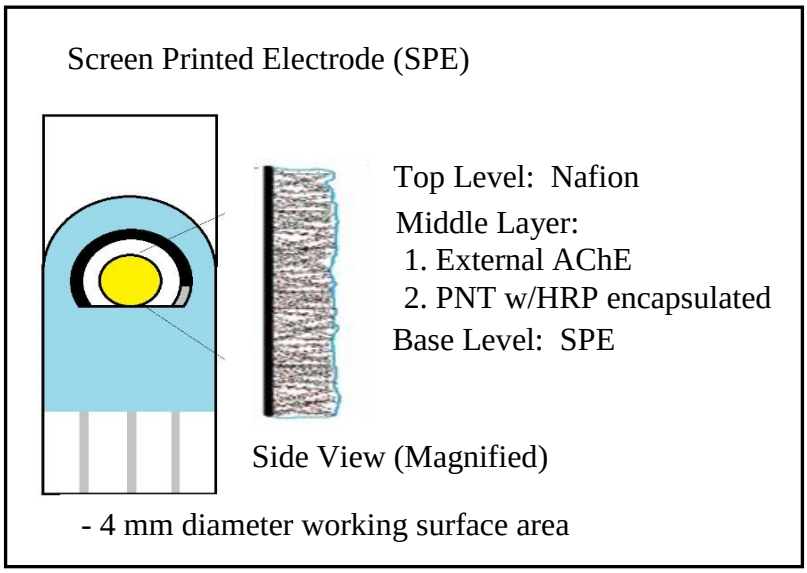
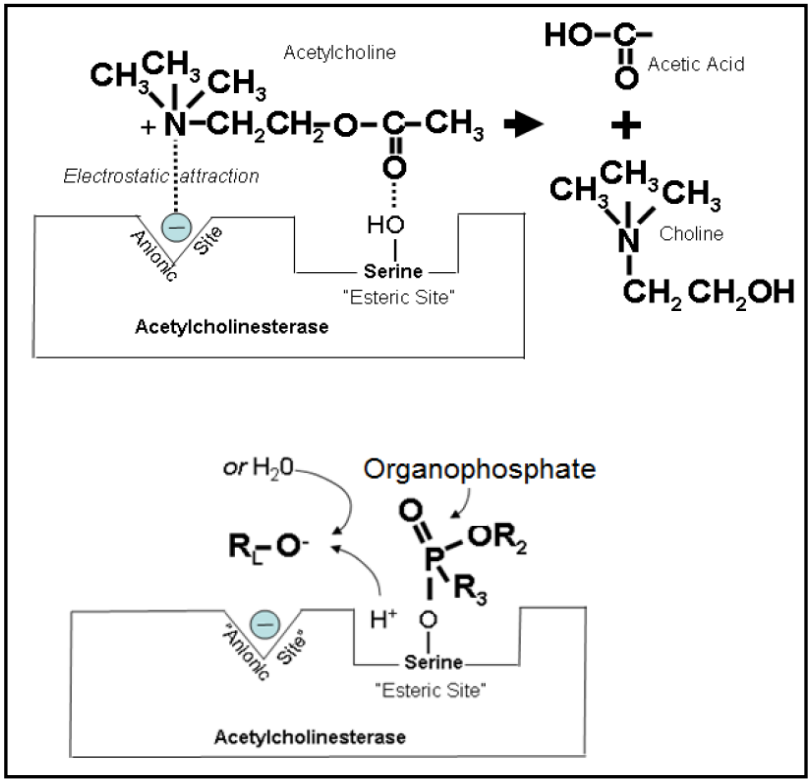


Figure 1: Disposable Biosensor Construct (Baker, 2013)

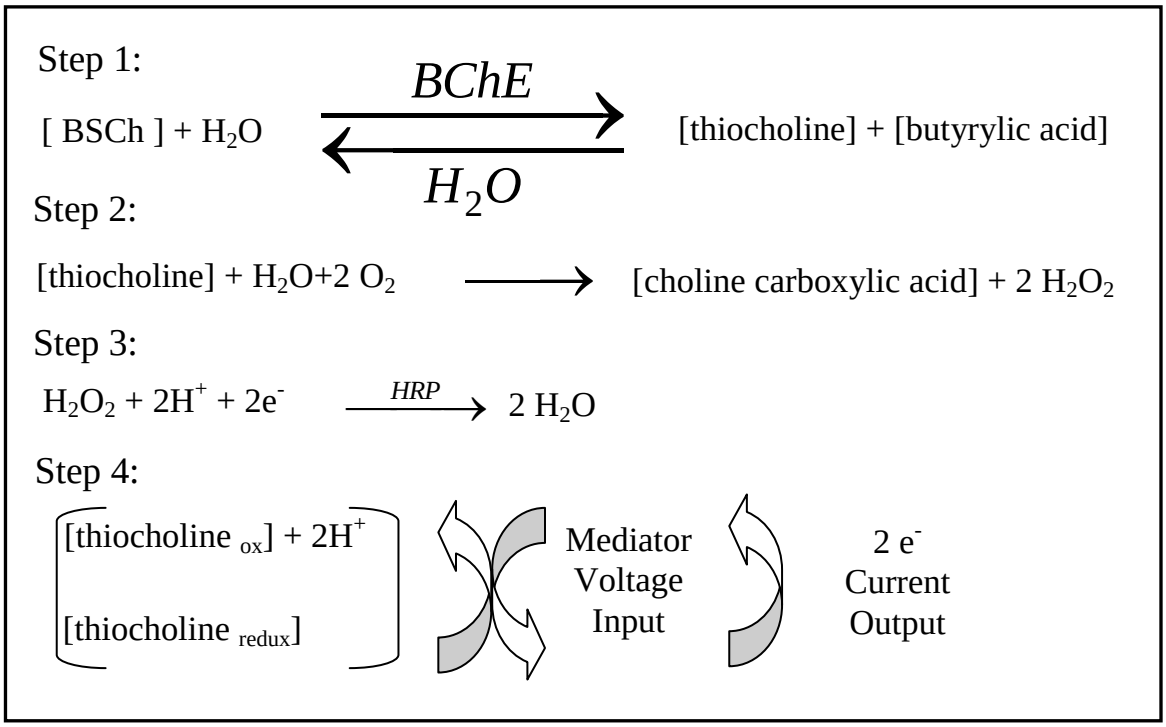


(Adapted from Baker, 2013)

Figure 2: Normal Acetylcholine Hydrolysis and Inhibition of AChE by OP

Arduini et al. (2007) demonstrated cost effective application of a Prussian blue-modified silver SPE using butyrylcholinesterase (BChE) enzyme and butyrylthiocholine (BSCh) as the substrate to detect the gas phase OPC nerve agents, Paraoxon, Sarin and VX. In the current study, we investigate the potential of fabricating a BChE-based biosensor on a reusable gold-SPE, using PNT, HRP, and Nafion to increase stability and sensitivity. The ability of the biosensor to measure malathion in the gas phase can then be evaluated.

The principle upon which a BChE-based biosensor works is depicted in Figure 3 (Andreescu et al., 2002; Andreescu and Marty, 2006). BChE catalyzes hydrolysis of BSCh (Step 1). The Step 1 reaction is inhibited by the presence of an OPC, because the OPC binds with the BChE enzyme. The intermediate breakdown of thiocholine in step two produces hydrogen peroxide (Andreescu et al., 2006). Hydrogen peroxide is



(Adapted from Andreescu, 2006)

Figure 3: BChE/BSCh enzyme electro-chemical reaction based biosensor

hydrolyzed in the presence of the HRP catalyst and is broken down into water. Thus, addition of HRP on the sensor increases sensor sensitivity by facilitating electron flow and allowing the reaction to take place at lower voltages (Park et al., 2012b; Baker, 2013).

To enhance enzyme function in biosensors, peptide nanotubes (PNTs) have been used (Park et al., 2010; 2012a; 2012b). The PNTs serve to protect enzyme activity, thereby increasing shelf-life and performance of the biosensor. Biosensor fabrication may also include application of a final top layer component. The top layer provides additional protection as well as acting as an adhesive to bind the PNTs to the electrode. Nafion, a Teflon-based stable polymer has been applied to bind PNTs (and their associated enzymes) to an electrode (Norouzi et al, 2010; Ren et al, 2012). When used in combination, the three materials: PNTs, HRP, and Nafion are used to: increase the contact area between the enzymes and the chemical compounds, protect the enzymes, and increase sensor sensitivity (See Figure 4).

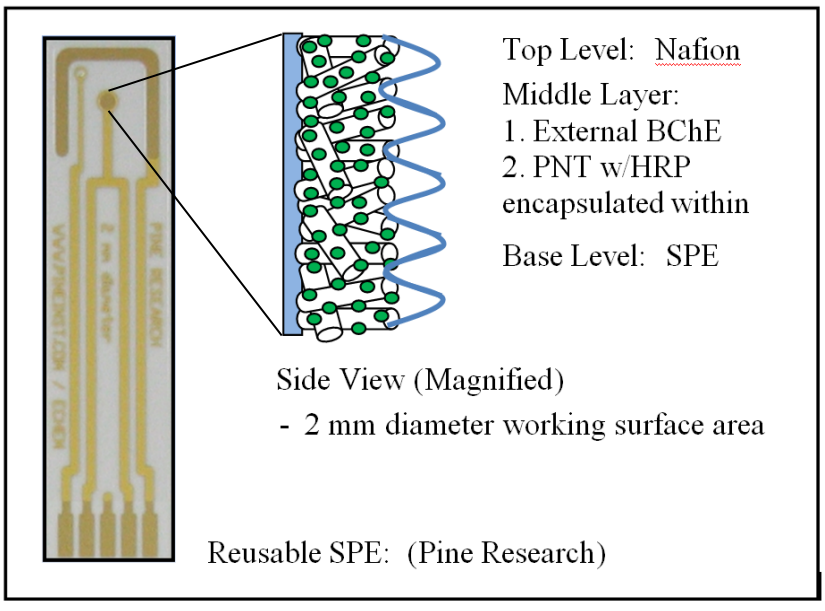


Figure 4: Biosensor Construct

The goal of this line of research is to create an inexpensive BChE-based biosensor capable of OPC detection and explore its performance characteristics: longevity (post fabrication shelf-life under laboratory conditions), sensitivity (limit of detection (LOD), precision, OPC concentration-inhibition response relationship), and reusability of the SPE (using commercially available off the shelf (COTS) reusable SPEs).

Materials, Equipment, and Methods

Materials

The B3128-1G, S-Butyrylthiocholine Chloride; C1057-1KU Butyrylcholinesterase From Equine Serum; P8250 – 5KU horseradish peroxidase (HRP); 180955-25G, Cellulose Acetate, 39.8 Wt. % Acetyl Content; 105228-25G, 1,1,1,3,3,3-Hexafluoro-2-Propanol, 99+%; A1542-250G, Ammonium Acetate Molecular Biology Reagent; P3786-1KG, Potassium Phosphate Dibasic, ACS Reagent, >=98%, and Malathion >95% were purchased from Sigma Aldrich (St. Louis, MO). ASCh and H-Phe-Phe-OH were purchased from Sigma Aldrich (St. Louis, MO), and stored at 4°C. Nafion© 117 solution (approx. 5%) was purchased from Sigma Aldrich (Allentown, PA). Deionized water was generated in the lab via reverse osmosis.

Gold screen-printed electrodes (SPE), model RRPE2001AU-6, with a 2-mm-diameter gold working electrode, the electrode-potentiostat interface cable, and the jacketed compact voltammetry cell were all purchased from Pine Research Instrumentation (Durham, NC).

Equipment

All electrochemical measurements were conducted using a Parstat 2273 Advanced Electrochemical System and PowerSuite ©Software from Princeton Applied Research. The SPE media were dried using AFIT supplied nitrogen gas. Peptide nanotubes (PNTs)

were agitated using a Cole-Parmer 8890 Sonicator. Experiments were completed using a Pine Research Instrumentation jacketed compact voltammetry cell. Malathion concentration was determined using an Agilent Technologies 6890N Network GC Systems model gas chromatograph mass spectrometer (GC/MS) (Baker, 2013).

Methods

PNTs were synthesized by dissolving 100 mg of H-Phe-Phe-OH in one ml of 1,1,1,3,3,3-hexafluoro-2-propanol. This mixture was swirled gently by hand for a few seconds and then placed in a sonicator for five minutes to ensure complete dissolution.

For some test configurations, to encapsulate the BChE inside the PNTs, one milliliter of PNT solution was dried overnight in a vacuum oven or ventilation hood with nitrogen gas applied. One milliliter of 50 mM, 7.4 pH phosphate buffer solution (PBS) containing one milligram BChE was then added to the PNT solution. Prior to selection and formal investigation into one compositional matrix, several fabrication processes were tried. While some involved a simple change in the layering order, others involved the use of cellulose acetate and no protective Nafion covering. Each compositional matrix was tested with the same protocol; qualitative performance criteria were used to select the compositional matrix with the highest prospects for continued research. Ultimately, the primary test configuration chosen for this research involved PNT encapsulation of HRP utilizing the encapsulation process described above. The PNT mixture was vortexed briefly and then incubated on a rotator in a temperature controlled environment at five Celsius, 30 rpm for one week. The PNT mixtures were kept refrigerated until needed for biosensor fabrication.

As an initial test of a reusable electrode, a Pine Research SPE with one fourth the working surface area of the SPE used by Baker (2013) was purchased and utilized

throughout the following experiments. The biosensors were prepared by first depositing 2.5µL of PNTs containing the encapsulated BChE on the working electrode, which was then allowed to dry in a hood at room temperature and pressure (average of 65°F and 745 mm Hg). Then, 2.5µL of 1000 U/mL HRP was deposited on top of the PNTs and allowed to dry. Next, 2.5µL of Nafion was deposited and allowed to dry.

Vapor concentration for the sensitivity tests was adjusted by injecting a known volume of gas saturated at room temperature with malathion (vapor pressure = 25 ppbv), into a 40 ml vial purged with nitrogen at constant temperature. The same set of SPEs was used for every subsequent sensitivity experiment. After each sensitivity experiment at a specific malathion concentration, the SPEs were initially cleaned using methanol. The CV instrument was utilized with the SPE immersed in a dilute acetic acid solution to further clean the SPE until the CV "finger print" plot demonstrated a baseline signature. To evaluate SPE reusability, for each sensitivity experiment, the same SPEs were re-fabricated with new enzyme layering prior to exposure to a different malathion concentration. The test protocol first involved electrode placement into a 20ml CV flask filled with a 7.4 pH PBS and CV measurement number one (CV#1) was taken. CV#1 was used to verify the condition of the electrode prior to conducting the experiment. After placement in the PBS solution, the CV#1 scan of the electrodes had a typical shape. In the event the scan of an electrode was atypical, that electrode was not used until it was cleaned, refabricated, and CV#1 rerun. After CV#1, the electrodes were then inserted into a 20ml CV flask containing one millimolar concentration BSCh, and CV measurement number two (CV#2) was taken. The electrodes were then transferred to a 40 ml vial that had been purged with nitrogen and then injected with a fixed concentration of malathion gas vapor, and CV measurement number three (CV#3) was taken. The characteristics of the CV#3 scan were

compared to earlier CV#3 scans to decide if subsequent test data from that electrode were valid. Finally, the biosensor was reinserted into the BSCh containing vial to obtain a post malathion CV measurement four (CV#4). In Figure 5, lines A and B represent pre-malathion, CV#2, and post malathion, CV#4, respectively.

Weekly longevity experiments involved simultaneous preparation of several sets of biosensors that were fabricated as described above and stored dry at room temperature (65 degrees Fahrenheit) in a dark cabinet. At weekly intervals, one set of sensors underwent the same test protocol as described for the sensitivity experiment except the OPC concentration remained constant at 25 ppbv malathion in a 40ml vial when CV#3 was made.

Results

Analysis of CV signatures for the BChE-based biosensor are notably different between pre- and post malathion exposure (Figure 5, lines A and B). The lines in figure 5 are representative of a single biosensor electrode test. Line A (CV#2) represents a single CV scan of the fabricated biosensor electrode prior to malathion exposure. Line B (CV#4) represents a post malathion exposure scan. Line C is the difference in current between line B and line A.

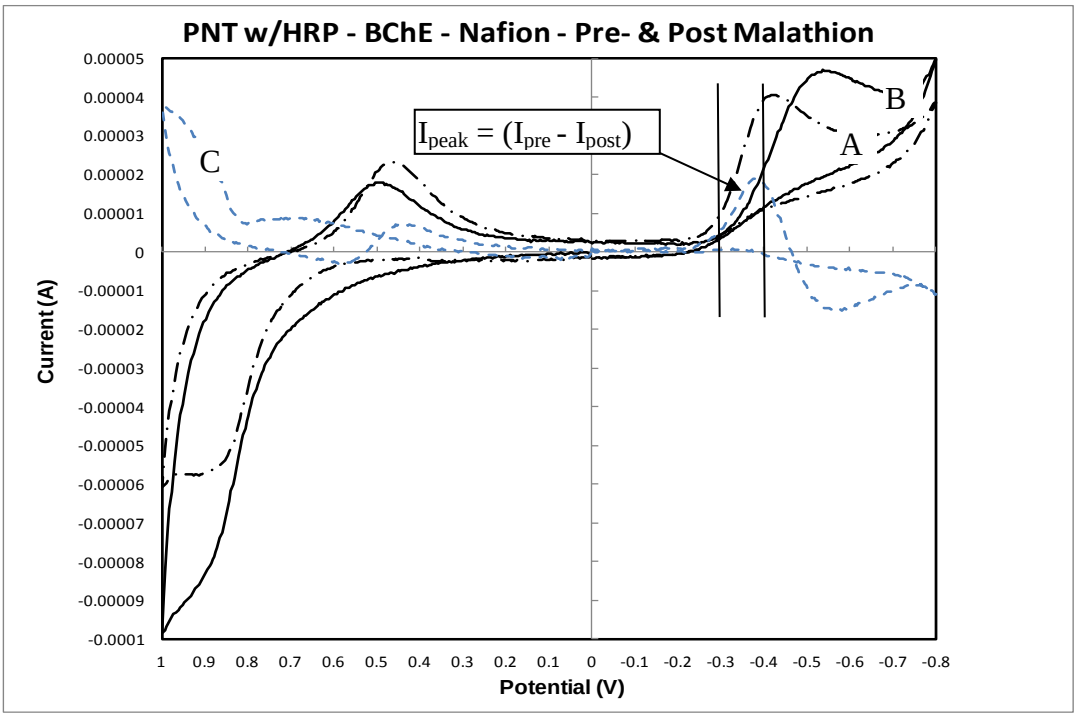


Figure 5: Representative CV Data for PNT w/HRP - BChE - Nafion Composition Matrix: Line A is CV#2 (I_{pre}), Line B is CV#4 (I_{post}), and Line C is (I_{pre} - I_{post})

As shown in Figure 5, this fabricated BChE-based biosensor example has a line C "finger print" region from -0.30 through -0.40 volts (indicated by the two vertical lines on the plot), where a distinctive peak for line C is realized. For each sensitivity experiment a plot similar to Figure 5 was completed. A visual inspection of the plot focused on Line C, looking for a characteristic peak within the -0.3 and -0.40 volts range. This signature "finger print" peak on Line C indicated the biosensor was operating within normal parameters. The line C peak (within the -0.30 to -0.40 voltage range) was used to calculate inhibition, as described below.

For each biosensor tested, the percent inhibition was calculated using the following equation:

$$\text{Percent Inhibition} = \frac{(I_{pre} - I_{post})}{I_{pre}} \text{ X } 100 \quad (1)$$

where: I_{pre} and I_{post} are current (A) values at a particular potential (V). .

As noted above, Line C is the numerator in Equation 1. Thus, for each biosensor test, a peak in Line C was located within the potential range -0.3 and -0.4 volts and Equation 2 applied:

$$\text{Percent Inhibition} = \frac{(I_{peak})_{\text{line C}}}{(I_{pre})_{\text{line A}}} \text{ X } 100 \quad (2)$$

where: (I_{peak}) is the current at the peak of line C (within the specified potential range)

(I_{pre}) is the current value on line A determined from the same voltage input as Line C's current peak output.

Note that the CV scan lines in Figure 5 actually represent a smoothed fit to multiple data points. To calculate an average percent inhibition from a particular CV scan, multiple pairs of data points along lines C and A (each pair of points corresponding to a particular voltage) were used in Equation (2) to calculate a percent inhibition at that voltage. A minimum of three consecutive percent inhibition values were used to determine an average percent inhibition for each biosensor test. Each data point shown in Figure 6 represents this average percent inhibition for a biosensor test. Each data point shown in Figure 6 represents the final percent inhibition value for a validated BChE-based biosensor electrode test.

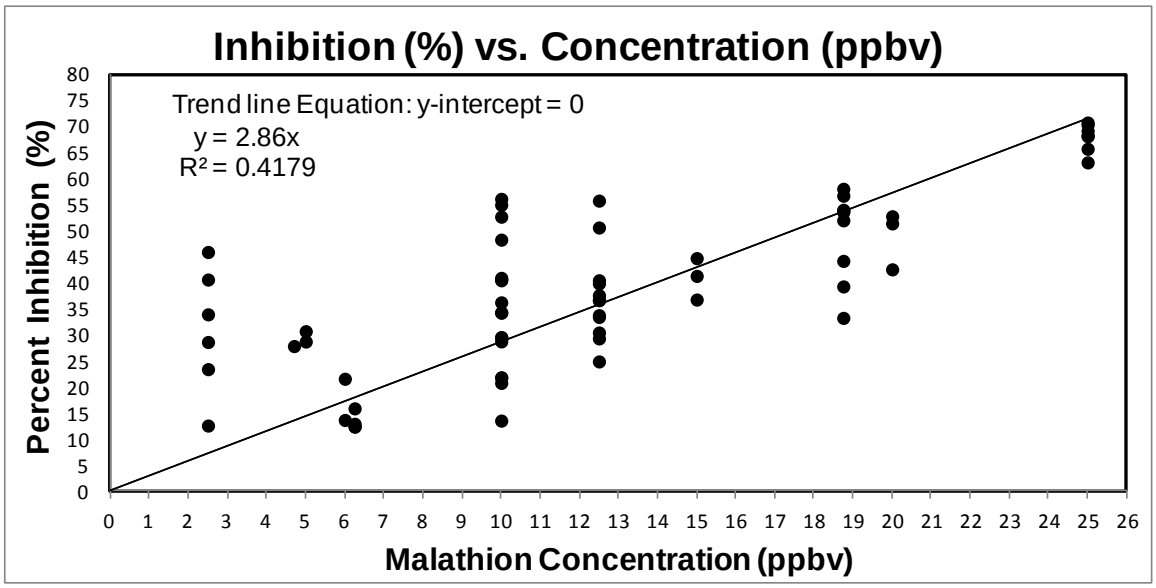


Figure 6: Sensitivity Test Data:

Note in Figure 6 that the linear correlation between percent inhibition and malathion concentration is relatively low. Looking at the figure, the low correlation appears due to the measurements made of malathion concentrations less than 6 ppbv. A new plot, Figure 7, was created using data only from concentrations 6 ppbv and higher. As seen in Figure 7, the linear correlation is markedly improved. Thus, if we assume the limit of detection of the biosensor for malathion is 6 ppbv, we see that there is a linear relation between percent inhibition and malathion concentration for the range of concentrations between the LOD and the vapor pressure of malathion (25 ppbv).

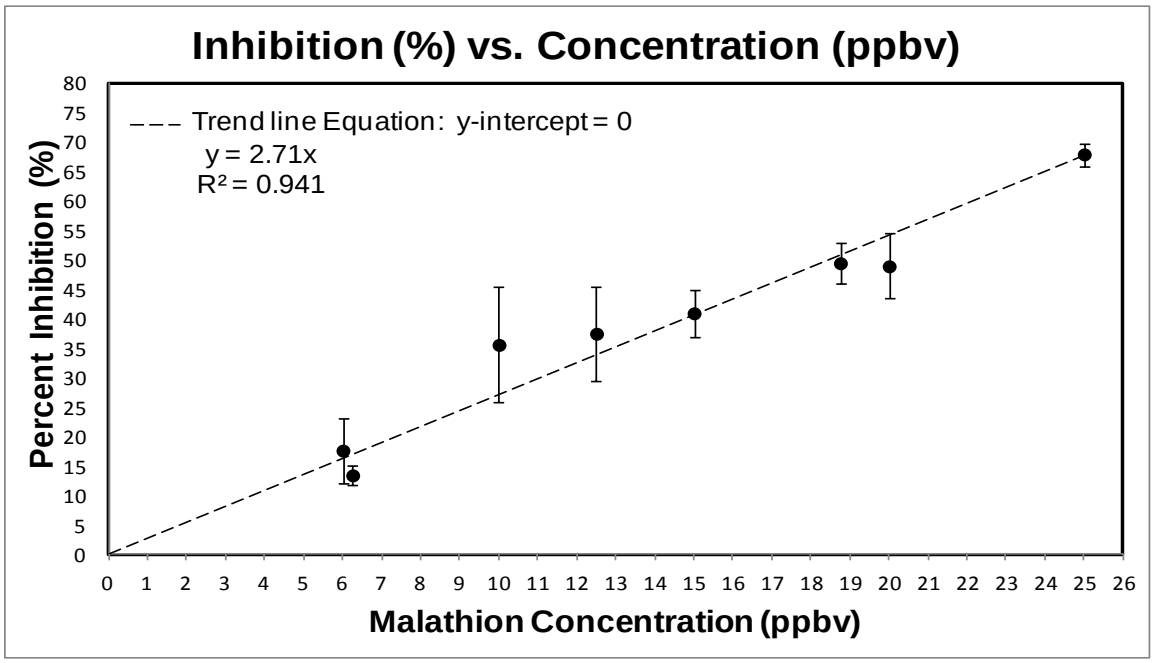


Figure 7: Sensitivity Test Data:
Simplified plot depicting only malathion concentrations above 6 ppbv; points represent average percent inhibition for multiple CV runs and "whiskers" represent the standard deviation.

In Figure 8, biosensor current and percent inhibition is shown for the longevity experiment. The longevity test data indicate that both biosensor current and percent inhibition should be considered when determining shelf life. Noting that the current responses were minimal after week 6, another graph was plotted, Figure 9, using the first six weeks of data and including the 25 ppbv malathion concentration data point from Figure 7 to represent week zero.

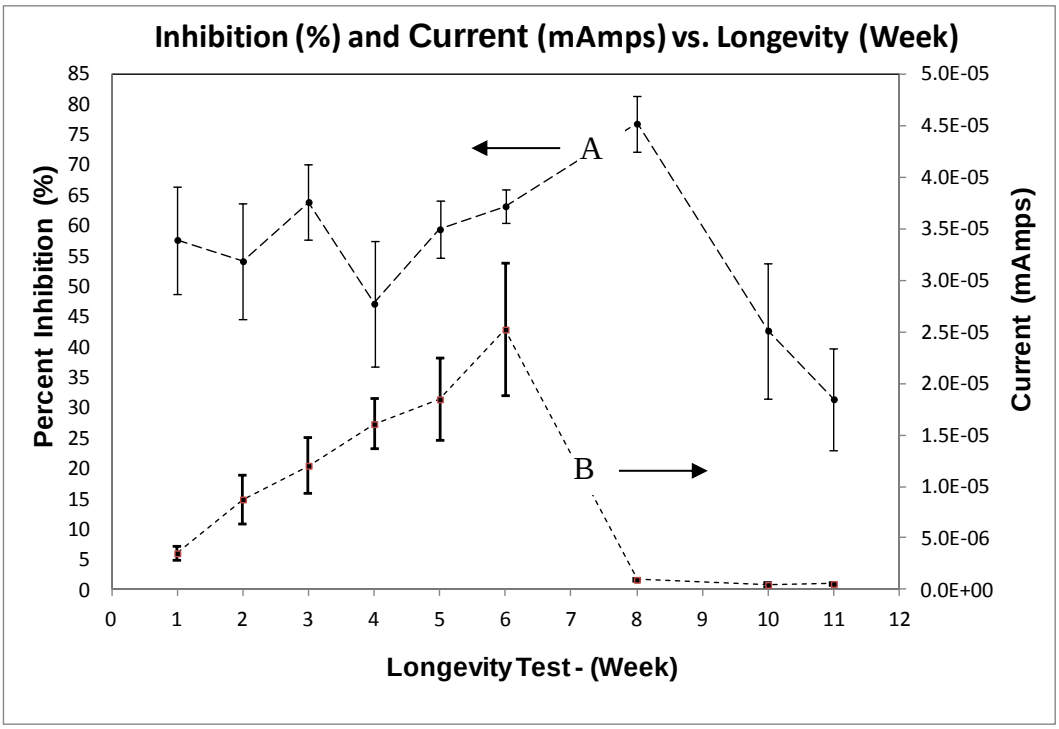


Figure 8: BChE/BSCh Longevity Test Data Showing Inhibition Measured after Biosensor Exposure to 25 ppbv Malathion

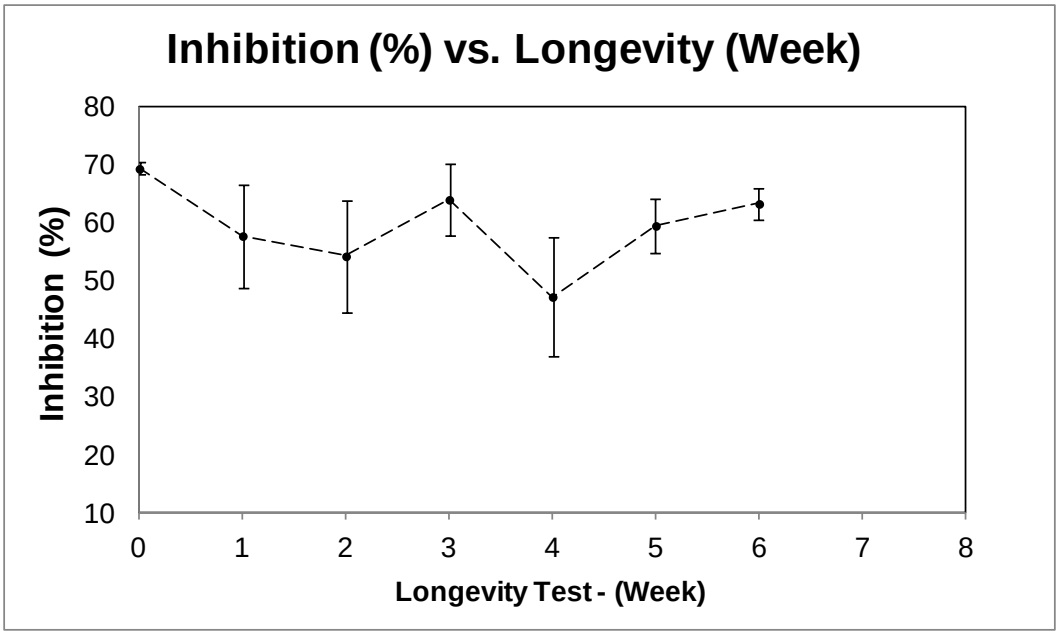


Figure 9: BChE/BSCh Longevity Test Data for First Six Weeks Showing Inhibition Measured after Biosensor Exposure to 25 ppbv Malathion

Discussion

Regarding the sensitivity tests, OPC detection using a BChE-based biosensor is demonstrably improved when compared to Baker's AChE-based biosensor. At 6 ppbv, the LOD was lower than the LOD of 12 ppbv achieved with the AChE-based sensor, and the working surface area of the reusable electrode was one fourth the size of the disposable electrode used for the AChE studies. While others have also investigated application of cholinesterase-based biosensors to detect gas phase nerve agents and other pesticides (e.g., Arduini et al., 2007; 2010; Arduini and Pelleschi, 2012b) those studies involved different compounds, and the results are not directly comparable to the results of this study. However, the performance of the BChE-based malathion detector with a gas-phase LOD of 6 ppbv developed in this study provides evidence that such a detector may have potential applications for chemical warfare agent detection.

For the longevity tests, Figure 8 shows a marked decrease in current after week 6 for a biosensor stored at room temperature. Baker (2013) demonstrated similar results, with an AChE-based biosensor's performance degrading significantly between 45 days and 60 days after dry storage at 4 Celsius. The results in the current study are also comparable to the results of fifty day longevity experiments conducted by Arduini et al. (2010) where different OPCs (VX, Sarin, and Paraoxon) were utilized.

Conclusion

This research demonstrated BChE/BSCh biosensors can be constructed to detect gas phase concentrations of malathion well below its vapor pressure of malathion. Based on percent inhibition of the BSCh hydrolysis reaction, quantitative measurements can be made for malathion concentration between the LOD of 6 ppbv and the vapor pressure of

25 ppbv. The sensors can be fabricated and stored at room temperature for up to six weeks with minimal performance degradation.

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Bibliography

The references of this article are combined with the thesis following the appendices.

III. Conclusion

Chapter Overview

This chapter provides a synopsis of findings in relation to the original research goals outlined in Chapter 1. However, due to length constraints for this manuscript, the scholarly article does not include some relevant discussion. For additional details refer to Appendices A, B, and C. This chapter also explores the limitations and the significance of this research, as well as provides suggestions for future research.

Review of Findings

The discussion below provides a review of the findings with regard to the research objectives presented in chapter one:

1. Over what concentration range is the inhibition of the hydrolysis reaction proportional to the concentration of the model OPC, malathion? What was the detection limit (LOD) for malathion?

Inhibition of the BSCh hydrolysis reaction was found to be linearly proportional to malathion concentration between the vapor pressure of malathion (25 ppbv) and the LOD (6 ppbv).

2. What was the post fabrication shelf-life for the BChE-based biosensor stored at room temperature?

Based on experimental data, shelf-life, was about 6 weeks. This compares with Baker's (2013) AChE-based biosensor, which exhibited performance degradation after 45 days after cold, dry storage at 4 Celsius.

3. Could the SPEs used to construct the biosensor be reused? Yes.

Significance of Research

The ultimate goal of this research is to produce a working sensor that can be commercialized for OPC detection. A small, wearable, simple detector could be used by agriculture workers to protect them from pesticide exposure or by soldiers who may be exposed to chemical warfare agents on the battlefield. This research successfully demonstrated fabrication of an OPC biosensor that would reliably detect the presence of a model OPC at concentrations well below the OPC vapor pressure. This research demonstrated that the biosensor could be stored after initial fabrication for up to six weeks without the need for refrigeration.

Limitations

OPC detection utilizing a BChE-based sensor involves reversibility with various compounds. The reversibility of certain OPCs is variable based on the affinity of the exposure compound to BChE. The affinity characteristic for each OPC lies along a spectrum of values. In other words, while malathion was selected and used to develop this BChE-based sensor, other OPCs will have a different inhibition response. Until this BChE-based biosensor is actually tested using more pesticides and warfare agents, extrapolating the data to other compounds is problematic. Despite the encouraging success of this research, the inability to adequately extrapolate to more dangerous compounds is a definitive limitation of this research.

Because the bond between malathion and BChE is reversible, data gathering proved challenging. The percent inhibition measured in the CV test varied, depending on the time that elapsed between exposing the sensor to malathion and the CV measurement.

With two technicians working in tandem, it was possible to minimize this time and make more consistent measurements. However, the standard deviation for the measurements in this research was compared to Baker's (2013) AChE-based research and, it was demonstrably lower than this baseline. As will be discussed in the next section, the BChE's reversible binding property may be exploited for development of a reusable biosensor.

Future Research

Development of a reusable biosensor:

Unlike Baker's AChE-based biosensors which were disposable after a single use, a BChE-based biosensor may be reused after exposure to an OPC, due to the equilibrium seeking characteristic of the chemical process. Due to the reversible nature of the BChE – malathion reaction, the ability to record inhibition between CV#2 and CV#4 during the experiment proved challenging. A test protocol needs to be developed that can demonstrate consistency between successive malathion exposures to the same BChE biosensor without a re-fabrication of the SPE working surface area.

- Repeat/Confirm analysis: BChE and AChE have comparable characteristics. For true comparison the AChE-based biosensor needs to be conducted on the reusable electrodes. Along with this, additional repeat studies on room temperature shelf-life should incorporate monitoring of both temperature and humidity variations.
- Investigate a reusable BChE-based biosensor: Unlike previous research which utilized disposable electrodes, this research successfully demonstrated electrode reusability. While there is tremendous savings associated with reuse of gold plated

SPEs rather than single use, disposable SPEs, research and development is still costly.

Recommendation: Apply the methods in this study to investigate whether the inhibition response of a BChE-based biosensor will remain stable over multiple uses.

Potential biosensor performance improvements:

Based on the "no protective layer" test results carried out (Appendices B and C), sensors without Nafion utilizing BChE do require a protective layer such as Nafion or cellulose acetate. The "no-Nafion" test affirmed that an additional protective layer such as Nafion has an important role in OP detection. It was postulated that switching from Nafion to cellulose acetate, used as the protective layer over HRP-treated PNT-BChE modified electrode, would assist in immobilizing the enzyme on the PNTs and improve performance (Baker, 2013; Arduini & Palleschi, 2012b). Arduini et al. (2012a) suggested using cellulose acetate instead of Nafion to further enhance biosensor performance. Cellulose acetate may improve biosensor longevity. Arduini and Amine (2014) have also indicated longevity, shelf-life success using a glutaraldeyde, bovine serum albumin (BSA), and Nafion biosensor composition matrix. Instead of measuring longevity in weeks, Arduini and Amine (2014) expressed it in months while storing at room temperature and in dry conditions. They indicated working stability is governed by pH, temperature, and matrix composition.

Based on experimental testing, an inhibition measurement consistent with the literature review and previous AFIT research indicated that enhanced inhibition was likely, due to HRP and hydrolysis of hydrogen peroxide into water. Significant biosensor longevity and sensitivity was achieved with chemically active HRP. While fabricated

BChE biosensors have lasting performance without cold, dry storage; future research may be consistent with Baker's 2013 AChE-based experiments. Additional research effort may discover that refrigeration enhances BChE-based longevity. Additional longevity testing with more robust environmental monitoring or further modification of the data gathering test procedure may be able to extend biosensor serviceable life and/or differentiate which environmental control variables will extend improved performance. The following is a list of some potential areas of future research:

- Vary the biosensor composition matrix (layering of PNT, catalyst, enzyme) and test sensor parameters: Investigate the impact derived from keeping the enzyme fixed while changing the order of the fabrication layering process. Room temperature, humidity, and compositional matrix may all impact biosensor sensitivity and longevity.
- Explore biosensor development with alternative OPC detecting enzyme compounds: Arduini and Amine (2014) noted several enzymes can be used in biosensor construction. Among them are peroxidase, tyrosinase, laccase, and glucose oxidase. From 2006 through 2012 research into these enzyme compounds has been limited. Investigating the application of these enzymes for OPC detection appears warranted.

It is evident that a small, reusable BChE-based electrode can be re-fabricated to detect low concentration gas phase malathion and subsequently utilized after several weeks in storage under standard laboratory conditions. The next logical step beyond continued refinement of a reusable SPE is extending reusability of the actual fabricated biosensor.

Recommendation: Carry out additional research to further characterize and optimize the variables that extend longevity and reusability.

Appendix A. Experiment Methodology

A-1 Sensitivity Experiment:

Vapor concentration was adjusted by injecting a known volume of gas saturated with malathion at its vapor pressure, a known concentration of 25 ppbv, into a 40 ml vial purged with nitrogen at constant temperature. The resulting concentration was calculated after equilibration was achieved using Raoult's law of thermodynamics.

The biosensors were prepared by first depositing 2.5µL of PNT/HRP, which was then allowed to dry in a hood at room temperature and pressure (average of 65°F and 745 mm Hg). Then, 2.5µL of 1000U/mL BChE was deposited on top and allowed to dry. Finally, 2.5µL of protective Nafion was deposited and allowed to dry. The biosensor was placed into a voltammeter flask filled with 7.4 pH phosphate buffer (PB) solution, and cyclic voltammetry (CV) measurements were taken. The biosensors were removed from the PB solution and inserted into an identical vial with PB solution containing 1 mmol BSCh, and CV measurements were taken again. The electrodes were then immediately transferred into a vial purged with nitrogen and replaced by a known concentration of malathion vapor. Finally, the biosensor was reintroduced to the BSCh solution and a final CV test was administered. The BSCh CV results from pre-malathion CV test and post malathion CV exposure test were compared. Inhibition was calculated as the difference between measurements and the pre-malathion current at each recorded voltage measurement.

Table A-1: BChE/BSCh, Malathion Sensitivity Data

	Percent Malathion Concentration – Sensitivity Summary Data												
Test Date	5 Sep	3 Oct	30 Sep	2 Oct	27 Sep	21 Oct	4 Oct	16 Oct	4 Oct	2 Oct	1 Oct	30 Sep	15 Oct
Percent	100%	80%	75%	60%	50%		40%		25%	24%	20%	18.8%	10%
(ppbv)	25	20	18.75	15	12.5		10		6.25	6[1]	5[2]	4.7[7]	2.5
Electrode													
1	70.8[3]	-	44.2	36.8	33.5	37.6	55.0	36.3	15.9	-	-	-	45.9
2	-	51.4	39.3	-	36.7	36.7	52.7	14.0	13.0	13.7[1]	-	-	34.0
3	-	42.6	33.3	41.3	50.6	33.8	56.1	29.6	12.5	-	-	-	40.6
4	70.4	52.8	1.47[4]	44.8	40.5	29.3	48.3	21.9	12.4	-	-	-	28.7
5	69.2	-	6.86[4]	-[5]	30.5	39.9	41.0[6]	20.8	-[5]	-	28.7	-	23.4
6	68.3	-	-	-	55.8	24.9	21.8[6]	34.4	-[5]	21.6[1]	30.7	27.9[7]	12.6
Average	69.3	48.9	38.9[8]	41.0[9]	41.3	33.7	45.8	26.1	13.4	17.6	29.7	-	30.9
Std Dev	1.04	5.53	5.48[8]	3.99[9]	9.97	5.64	13.0	8.69	1.67	5.59	1.40	-	12.0
Inhibition Summary	67.6	44.6	37.2	40.3	40.3	33.5	45.1	25.8	12.4	-	-	-	31.1
Potential (V) (max)	-0.34	-0.49	-0.32	-0.33	-0.32	-0.32	-0.32	-0.32	-0.39	-	-	-	-0.32
Potential (V) (min)	-0.34	-0.50	-0.33	-0.34	-0.34	-0.34	-0.33	-0.34	-0.41	-	-	-	-0.33

Note 1: Exposure concentration standard was 6 ppbv: 25 ppbv*(16/40)*(24/40).
Note 2: Exposure concentration was 5 ppbv: 25 ppbv*(8/40), for these electrodes.
Note 3: Electrode 1 utilized a 40 ml malathion sample vial at 100% concentration, 25 ppbv, and was tested on 1 Oct.
Note 4: For the 75% malathion exposure electrodes 4 & 5 were excluded from summary data. An error occurred during the process used to make the exposure 75% vial concentrations for electrodes 4 and 5.
Note 5: Results for this electrode were non-consistent and unusable.
Note 6: Electrode 5 utilized PNT/HRP formula that was 1 month old, electrode 6 utilized a formula that was 2 months old.
Note 7: Electrode 6 was prepared with a concentration of 4.7 ppbv malathion, 25ppbv *(16/40)*(30/40).
Note 8: Average calculation and standard deviation were calculated using electrodes 1, 2, and 3.
Note 9: Average calculation and standard deviation were calculated using electrodes 1, 3, and 4.

Table A-2: BChE/BSCh, Malathion Sensitivity Data (Continued)

Percent Malathion Concentration – Sensitivity Pooled Standard Deviation Summary Data[1]									
Test Date	5 Sep	29 Oct	30 Sep	3 Nov	27 Sep	21 Oct	4 Oct	16 Oct	21 Oct
Percent	100%		75%		50%		40%		
(ppbv)	25		18.75		12.5		10		
Electrode									
1	70.8[4]		44.2	52.0	33.5	37.6	55.0	36.3	34.3
2			39.3	56.7	36.7	36.7	52.7	14.0	28.8
3			33.3	58.1	50.6	33.8	56.1	29.6	40.0
4	70.4	68.2		53.6	40.5	29.3	48.3	21.9	29.6
5	69.2	65.7		53.9	30.5	39.9	41.0[6]	20.8	-[5]
6	68.3	63.1		54.0	55.8	24.9	21.8[6]	34.4	40.7
Average	69.3	65.7	39.0	54.7	41.3	33.7	45.8	26.1	34.7
Std Dev	1.04	2.52	5.48	2.24	10.0	5.64	13.0	8.69	5.62
Inhibition Summary	67.6	65.5	37.2	54.4	40.3	33.5	45.1	25.8	34.5
Potential (V) (max)	-0.34	-0.32	-0.32	-0.33	-0.32	-0.32	-0.32	-0.32	-0.32
Potential (V) (min)	-0.34	-0.35	-0.33	-0.35	-0.34	-0.34	-0.33	-0.34	-0.34
Pooled Average[2]	68.0		49.5		37.5		35.6		
Pooled Std Dev[3]	1.93		3.49		8.10		9.81		

Note 1: Pooled standard deviation is the square root of pooled variance.
Note 2: Pooled Average is the average of all samples taken in each set divided by the number of samples.
Note 3: Pooled Standard Deviation = $\text{sqrt}(S_p^2) = \text{sqrt}\{[(n_1-1)s_1^2+(n_2-1)s_2^2+ \ldots(n_k-1)s_k^2] / [n_{1+} n_{2+} \ldots n_k - k)]\}$
Note 4: Electrode 1 utilized a 40 ml malathion sample vial at 100% concentration, 25 ppbv, & was tested 1 Oct.
Note 5: Results for this electrode were non-consistent and unusable.
Note 6: Electrode 5 utilized PNT/HRP formula 1 month old, electrode 6 utilized a formula 2 months old.

A-2 Longevity Experiment:

All biosensors were prepared as specified in Table 3 and allowed to age. At one week intervals, the sensors were tested with 25 ppbv malathion vapor. Longevity experiment results are described in Appendix C and summarized in the following tables.

Table A-3. Longevity Exposure Experiment Preparation

Biosensor Surface Preparation:	Screen printed gold electrodes prepared with acetate solution. HRP encapsulated PNTs applied to surface and allowed to dry. BChE mid-layer added to surface, dried using Nitrogen gas. Nafion protective top-cover applied.
Biosensor Conditioning:	Initial immersion into phosphate buffer solution. CV #1 test applied. Immersed in BSCh solution and CV #2 applied.
Gas Phase Exposure:	Inserted into 25 ppbv Malathion gas vapor for two minutes. CV #3 applied.
Inhibition Measurement:	Immediately re-inserted into BSCh solution Applied CV #4 test.

Table A-4: BChE/BSCh Biosensor Longevity Data

	Week								
	0	1	2	3	4	5	6	8	10/11[3]
Electrode	Measured Inhibition (%)								
1		82.6	49.8	58.7	49.4	54.8	65.3	76.3	39.0
2	-	72.4	62.1	67.8	44.3	54.0	65.0	82.5	55.3
3	-	52.4	52.0	68.8	60.8	58.5	61.5	75.0	33.8
4	70.4	54.0	50.2	78.1	42.1	66.7	65.0	82.5	3.98
5	69.2	53.7	45.9	72.6	50.2	61.8	59.3	72.2	39.2
6	68.3	50.7	61.9	66.3	51.6	60.9	38.6	72.7	51.0
Average (%)	69.3	56.6	53.6	68.7	49.7	59.4	63.2	76.9	31.4
Std Dev	1.04	8.90	6.78	6.48	6.54	4.75	2.72	4.60	8.41
% Inhibition Summary	67.6	53.0	50.9	68.2	49.2	59.0	58.4	76.1	31.2
Potential (V) Range (max)	-0.34	-0.34	-0.37	-0.34	-0.35	-0.35	-0.34	-0.32	-0.32
Potential (V) Range (min)	-0.34	-0.34	-0.38	-0.37	-0.37	-0.37	-0.35	-0.35	-0.34

Note 1: CV curve analysis during malathion exposure indicated the electrode surface was coated with liquid PB; this resulted in an a-typical inhibition response of the biosensor.
Note 2: Analysis of CV curve electrode data indicates the sensor surface may have been disrupted during the test protocol resulting in erroneous data.
Note 3: Linear trend analysis indicated dysfunctional malathion inhibition detection after week 6. Biosensors 1, 2, and 3 were tested at week ten and electrodes 4, 5, and 6 were tested at week eleven.

Table A-5: BChE/BSCh Longevity Second Test with Pooled Standard Deviation

Electrode	Week[1]							
	1		2		3		4	
1	-[4]	69.7	49.8	63.3	58.7	66.2	49.4	47.6
2	72.4	64.7	62.1	32.2	67.8	61.7	44.3	43.2
3	52.4	48.9	52.0	56.3	68.8	-[4]	60.8	37.6
4	54.1	51.2	50.2	60.2	78.1	50.9	42.1	23.6
5	53.7	59.2	45.9	63.3	72.6	55.4	50.2	58.2
6	50.7	-[4]	61.9	53.0	66.3	56.8	51.6	57.3
Average (%)	56.6	58.7	53.6	54.7	68.7	58.2	49.7	44.6
Std Dev	8.90	8.78	6.78	11.7	6.48	5.89	6.54	13.0
% Inhibition Summary	53.0	58.0	50.9	53.8	68.2	58.2	49.2	44.4
Potential (V) Range (max)	-0.34	-0.33	-0.37	-0.34	-0.34	-0.32	-0.35	-0.32
Potential (V) Range (min)	-0.34	-0.36	-0.38	-0.36	-0.37	-0.35	-0.37	-0.35
Pooled Average[2]	57.7		54.2		63.9		47.2	
Pooled Std Dev[3]	8.84		9.58		6.22		10.31	

Note 1: The 1st column of each week is from the 1st longevity data set; the second is from a 4 week 2nd run.

Note 2: Pooled Average is the average of all samples taken in each set divided by the number of samples.

Note 3: Pooled Standard Deviation = $\sqrt{(S_p^2)} = \sqrt{\{[(n_1\text{-}1)s_1^2+(n_2\text{-}1)s_2^2+ \ldots(n_k\text{-}1)s_k^2] / [n_{1+} n_{2+} \ldots n_k - k)]\}}$

Note 4: CV curve was non-consistent and unusable.

Appendix B. Air Force Institute of Technology Thesis Results

B-1 Initial Experiment:

On 24 Jun 13, after receiving the initial chemical purchase order to conduct the thesis project, the first question that needed to be answered was whether the newly purchased Pine Research gold electrodes would achieve results similar to previous work using electrodes from another manufacturer. The newly purchased electrodes from Pine Research have a working surface area diameter one half the size. This reduced the surface area to one quarter the size previously used. The second question was whether switching BChE in lieu of AChE while still using the gas phase detection laboratory protocol developed by Peter Baker would still work.

As a test, two electrodes were prepared with 2.5 µL of PNTs encapsulating HRP. One electrode had 2.5 µL AChE applied to the working electrode surface. The other had 2.5 µL of BChE applied to the surface and allowed to dry. The electrodes were then coated with Nafion, allowed to dry, and then were tested. See Table B-1 for a synopsis of the biosensor development and test protocol.

Table B-1: AChE vs. BChE Using Reusable Electrodes

Surface Preparation:	Two electrodes cleaned with acetate cleaning solution. HRP encapsulated PNTs applied to surface and allowed to dry. AChE mid-layer added to electrode. BChE mid-layer added to electrode. Nafion protective top-cover applied. After each application, electrodes were dried using nitrogen.
Biosensor Conditioning:	Initial immersion into phosphate buffer solution. Cyclic voltammeter (CV #1) test applied.
Biosensor Test:	Immersed in BSCh or ASCh solution and CV #2 applied.

The electrode was immersed in PBS and a cyclic voltammeter test, CV#1, was taken. Then the electrode was removed from the solution and immersed in a PBS with one

millimolar BSCh or 1mmol ASCh solution and a CV was again taken. The CV "Finger print" for each electrode along with the difference is plotted in Figure B-1.

As expected, analysis of the CV signatures for BChE and AChE are notably different. To better understand the difference, a third line was developed that subtracted the ATCh result from the BSCh line. Finally, the "Difference" calculation was divided by the original corresponding BSCh data points to produce a standardized ratio. From this, two distinctive regions of potential interest were noticed. BChE was a distinctive CV fingerprint region at 0.7 – 0.5 Volts and another at -0.3 – -0.5 Volts setting it apart from AChE.

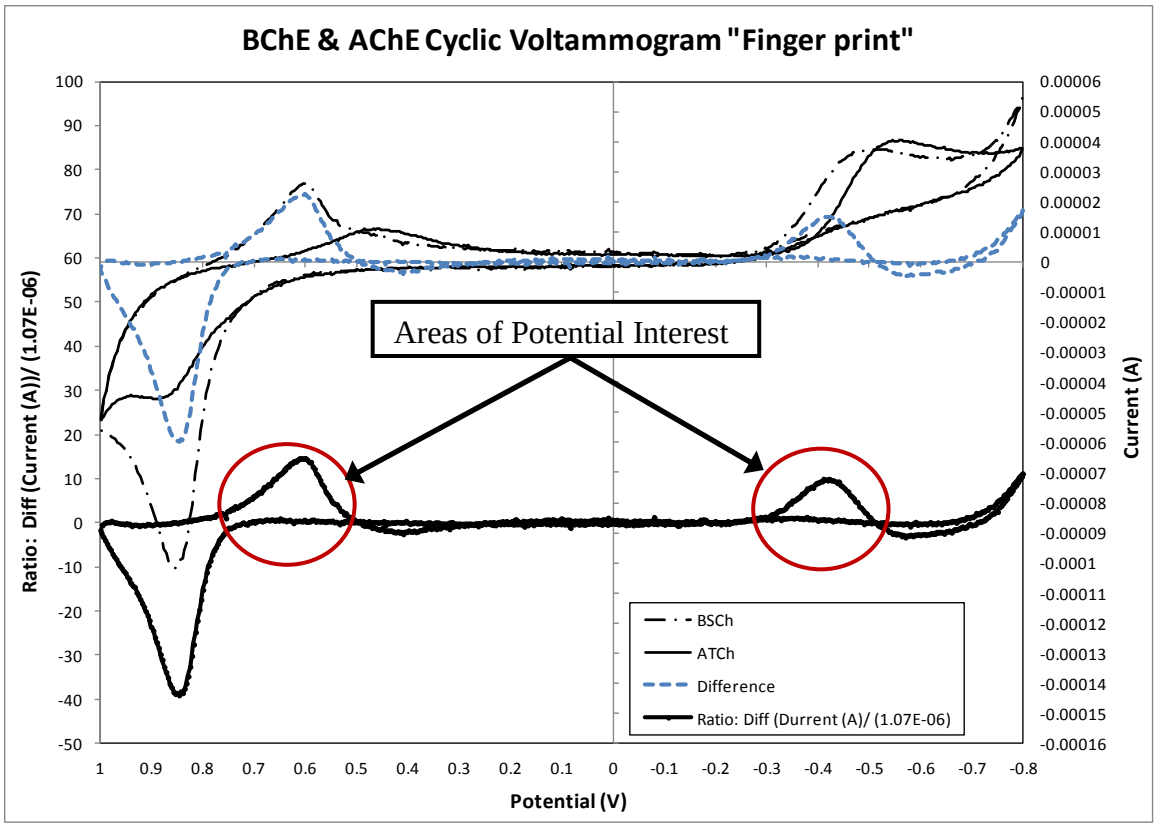

Figure B-1: Initial BChE and AChE Comparison Test

B-2 Establishing Baseline Experiment: (Repeating Peter Baker's Thesis Work)

On 8 Jul, six electrodes were prepared with 2.5 µL of PNTs encapsulating HRP. All six electrodes had 2.5 µL AChE applied to the working electrode surface and allowed to dry. The electrodes were coated with Nafion, allowed to dry, and then were tested. See Table B-2 for a synopsis of the biosensor development and test protocol. The electrode was immersed in PBS and a cyclic voltammeter test, CV#1, was taken. Afterward, the electrode was removed from the solution and immersed in a PBS with one millimolar ASCh solution and a CV was again taken. The electrode was inserted into a gaseous environment for two minutes of exposure containing 25 ppbv malathion gas and another cyclic voltammogram was taken. Finally, the electrode was reintroduced to the ASCh solution and another CV test was administered.

Table B-2: AChE Using Reusable Electrode Biosensor Preparation:

Surface Preparation:	Six electrodes cleaned with acetate cleaning solution. HRP encapsulated PNTs applied to surface and allowed to dry. AChE mid-layer added to three electrodes. Nafion protective top-cover applied. After each application, electrodes were dried using nitrogen.
Biosensor Conditioning:	Initial immersion into phosphate buffer solution. Cyclic voltammeter (CV #1) test applied. Immersed in ATCh solution and CV #2 applied.
Gas Phase Exposure:	Inserted into 25 ppbv Malathion gas vapor for two minutes. CV #3 applied.
Inhibition Measurement:	Immediately re-inserted into ASCh solution Applied CV #4 test.

Analysis of the CV signatures for ATCh are notably different between pre- and post malathion exposure. To better understand the difference, a third line was developed and plotted in Figure B-2 that subtracted the ATCh pre-malathion exposure from the post malathion exposure. Finally, the "Difference" calculation was divided by the original corresponding ATCh data points to produce a standardized ratio. From this, two distinctive

regions of potential interest were noticed. AChE was a distinctive CV fingerprint region at about 0.57 – 0.35 volts and another at -0.32 - -0.45 volts. After reviewing the areas of potential interest in Figure B-2, Table B-3 records the percentage of malathion inhibition measured on the right side "Area of Potential Interest" since it demonstrates a higher peak. Individual sensor and summary information was recorded.

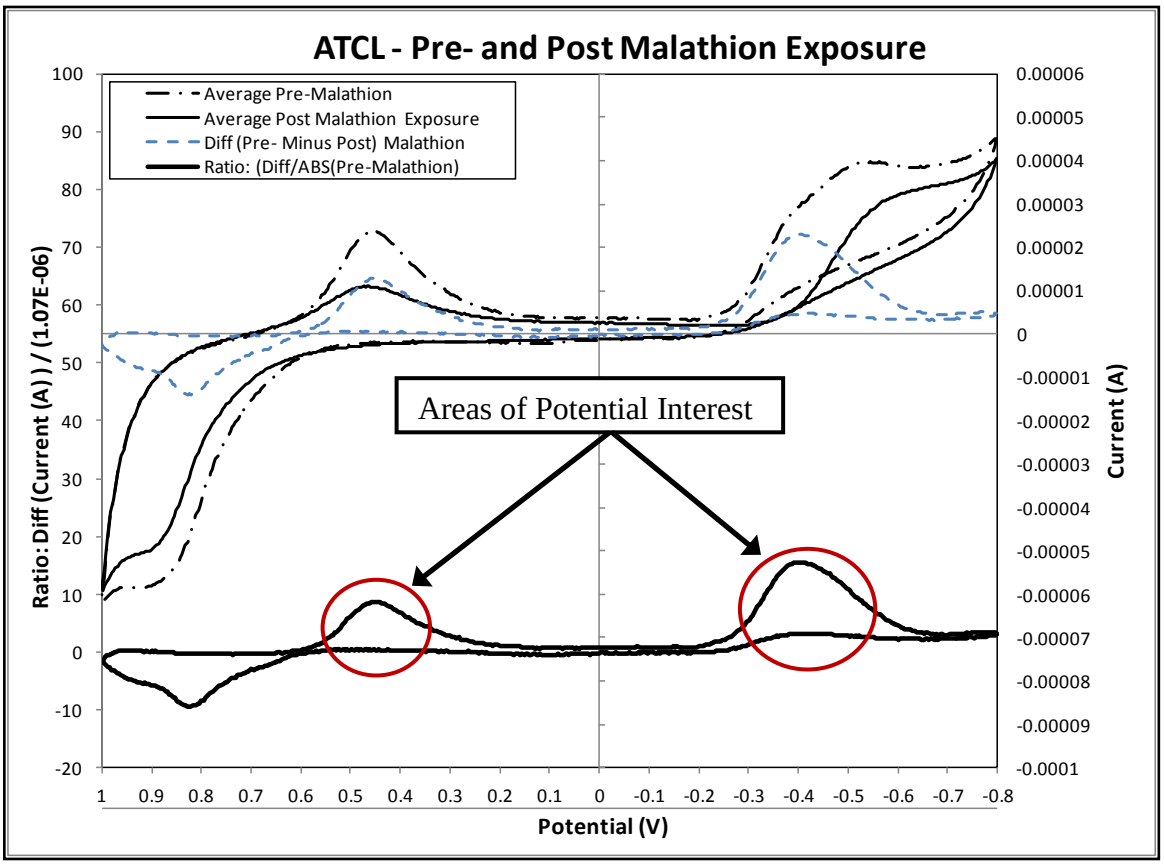

Figure B-2: Initial AChE and ATCh Biosensor Test

Table B-3: AChE Using Reusable Electrodes

Electrode #	Potential (V)	Inhibition (%)
1	-0.32 through -0.35	86.26
2	-0.32 through -0.36	89.57
3	-0.37 through -0.38	89.33
4	-0.39 through -0.41	75.95
5	-0.42 through -0.45	73.69
6	-0.42 through -0.44	69.41
Result Average	**-0.36 through -0.39**	**76.22**
Std Dev		**7.59**

B-3 Establishing BSCE – Cellulose Acetate Baseline:

On 22 Jul, six electrodes were prepared with 2.5 µL of PNTs encapsulating HRP. All six electrodes had 2.5 µL BChE applied to the working electrode surface and allowed to dry. The electrodes were coated with cellulose acetate, allowed to dry, and then were tested. See Table B-4 for a synopsis of the biosensor development and test protocol. The electrode was immersed in PBS and a cyclic voltammeter, CV#1, was taken. Afterward, the electrode was removed from the solution and immersed in a PBS with one millimolar BChE solution and a CV was again taken. The electrode was inserted into a gaseous environment for two minutes of exposure containing 25 ppbv malathion gas and another cyclic voltammogram was taken. Finally, the electrode was reintroduced to the BSCh solution and another CV test was administered.

Table B-4: BChE Using Reusable Electrode Biosensor Preparation:

Surface Preparation:	Six electrodes prepared with acetate cleaning solution. HRP encapsulated PNTs applied to surface and allowed to dry. BChE mid-layer added to three electrodes. Cellulose acetate dissolved in acetone applied as top-cover. After each application, electrodes were dried using nitrogen.
Biosensor Conditioning:	Initial immersion into phosphate buffer solution. Cyclic voltammeter (CV #1) test applied. Immersed in ATCh solution and CV #2 applied.
Gas Phase Exposure:	Inserted into 25 ppbv Malathion gas vapor for two minutes. CV #3 applied.
Inhibition Measurement:	Immediately re-inserted into ATCh solution Applied CV #4 test.

Analysis of the CV signatures for BSCh are notably different between pre- and post malathion exposure. To better understand the difference, a third line was developed and plotted in Figure A-3 that subtracted the BSCh pre-malathion exposure from the post malathion exposure. Finally, the "Difference" calculation was divided by the original corresponding BChE data points to produce a standardized ratio. From this, one distinctive regions of potential interest was noticed. BChE with cellulose acetate has a distinctive CV fingerprint region at about -0.30 - -0.45 volts. After reviewing the area of potential interest in Figure B-3, Table B-5 records the percentage of malathion inhibition measured. Both individual electrode information as well as the summary curve was recorded.

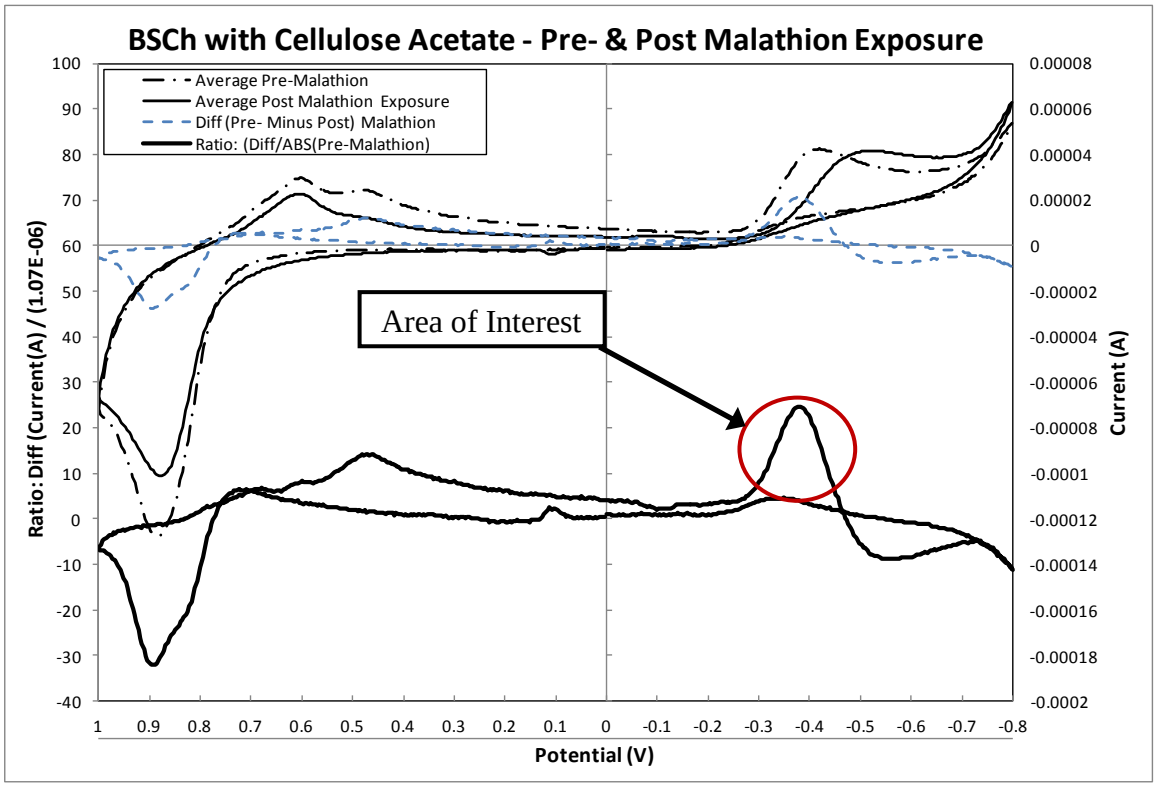

Figure B-3: Initial BChE and BSCh Biosensor Test w/Cellulose Acetate

Table B-5: BChE Inhibition Peak Using Reusable Electrodes

Electrode #	Potential (V)	Inhibition (%)
1	-0.33 through -0.35	82.71
2	-0.32 through -0.35	65.27
3	-0.32 through -0.34	72.79
4	-0.33 through -0.36	84.62
5	-0.33 through -0.37	56.18
6	-0.35 through -0.36	65.53
Result Average	**-0.33 through -0.35**	**70.56**
Std Dev		**11.93**

B-4 Establishing BSCE – Nafion Baseline through Experimentation:

On 8 Aug, two electrodes were prepared with 2.5 µL of PNTs encapsulating HRP. Both electrodes had 2.5 µL BChE applied to the working electrode surface and allowed to dry. The electrodes were top coated with Nafion and then were tested. See Table B-6 for a synopsis of the biosensor development and test protocol. The electrode was immersed in PBS and a cyclic voltammeter, CV#1, was taken. Afterward, the electrode was removed from the PBS and immersed in a PBS with one millimolar BChE solution and a CV was again taken. The electrode was inserted into a gaseous environment for two minutes of exposure containing 25 ppbv malathion gas and another cyclic voltammogram was taken. Finally, the electrode was reintroduced to the BSCh solution and another CV test was administered.

Table B-6: BChE Using Reusable Electrode Biosensor Preparation:

Surface Preparation:	Two electrodes prepared with acetate cleaning solution. HRP encapsulated PNTs applied to surface and allowed to dry. BChE mid-layer added to three electrodes. Nafion dissolved in DI water applied as top-cover. After each application, electrodes were dried using nitrogen.
Biosensor Conditioning:	Initial immersion into phosphate buffer solution. Cyclic voltammeter (CV #1) test applied. Immersed in BSCh solution and CV #2 applied.
Gas Phase Exposure:	Inserted into 25 ppbv Malathion gas vapor for two minutes. CV #3 applied.
Inhibition Measurement:	Immediately re-inserted into BSCh solution Applied CV #4 test.

Analysis of the CV signatures for BSCh are notably different between pre- and post malathion exposure. A third line was plotted in Figure A-4 that subtracted the BSCh pre-malathion exposure from the post malathion exposure. The "Difference" calculation was divided by a constant value to produce a standardized ratio which was plotted on the secondary axis. From this, one area of potential interest was noted. BChE with Nafion has

a distinctive CV "finger print" region at about -0.30 through -0.40 volts. After reviewing the area of potential interest in Figure B-4, Table B-7 records the percentage of malathion inhibition measured. Both individual electrode information as well as the summary curve were recorded. No voltage potential peak at 0.45V was observed.

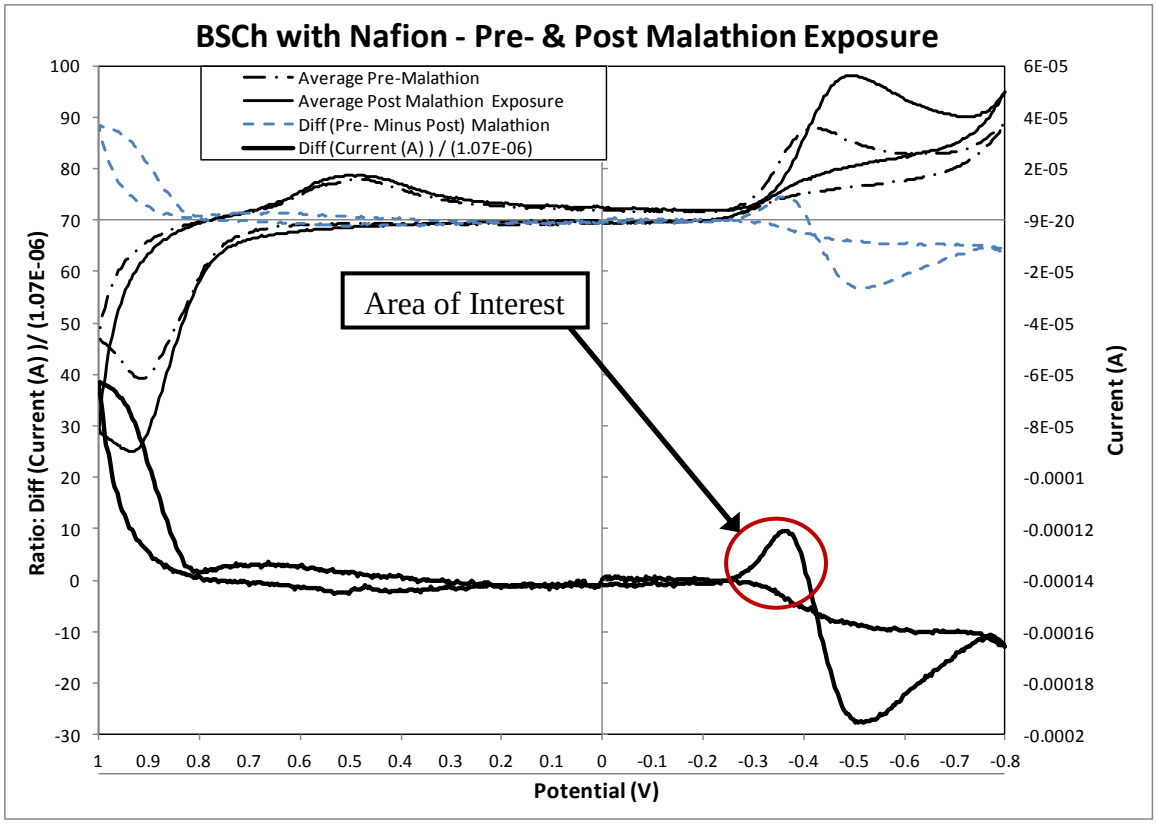

Figure B-4: Initial BChE and BSCh Biosensor Test with Nafion

Table B-7: BChE Inhibition Peak Using Reusable Electrodes

Electrode #	Potential (V)	Inhibition (%)
5	-0.32 through -0.35	63.08
6	-0.33 through -0.34	57.90
Result Average	**-0.32 through -0.34**	**60.28**

B-5 PNT – HRP - BSCE – Nafion Covered Biosensor Layering Variations:

On 16 Aug, six electrodes were tested after preparing them in three different layering configurations. Two electrodes were prepared with 2.5 µL of PNTs encapsulating HRP as the first layer followed by BChE and Nafion like the electrodes prepared on 8 Aug. The middle two electrodes were prepared using PNTs with no HRP encapsulation with BChE and Nafion. The last two electrodes were prepared with PNTs encapsulating BChE, HRP was added as the middle layer followed by the Nafion. See Figure B-5 for additional clarification. See Table B-8 for a synopsis of the biosensor development and test protocol.

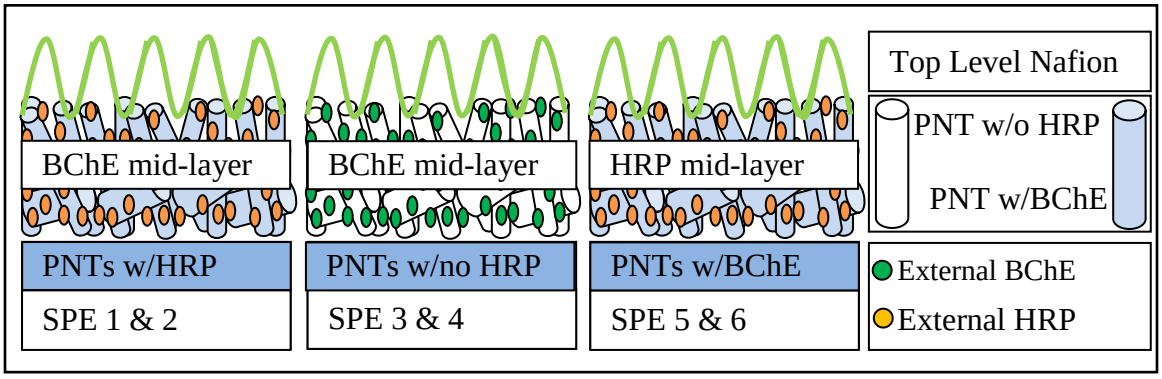

Figure B-5: Initial Layering Configuration with Nafion

Table B-8: BChE Using Reusable Electrode Biosensor Preparation:

Surface Preparation:	Six electrodes prepared in groups of 2 according to Fig. B-5. Nafion dissolved in DI water applied as top-cover. After each application, electrodes were dried using nitrogen.
Biosensor Conditioning:	Initial immersion into phosphate buffer solution. Cyclic voltammeter (CV #1) test applied. Immersed in BSCh solution and CV #2 applied.
Gas Phase Exposure:	Inserted into 25 ppbv Malathion gas vapor for two minutes. CV #3 applied.
Inhibition Measurement:	Immediately re-inserted into BSCh solution Applied CV #4 test.

Analysis of the CV signatures for BSCh are notably different between pre- and post malathion exposure. A third line was plotted in Figure B-6 that subtracted the BSCh pre-malathion exposure from the post malathion exposure. The “Difference” calculation was

divided by a constant value to produce a standardized ratio which was plotted on the secondary axis. From this, one area of potential interest was noted. BChE with Nafion has a distinctive CV "finger print" region at about -0.30 through -0.40 volts. After reviewing the area of potential interest for electrodes 1 & 2 in Figure B-6, Table B-9 records the percentage of malathion inhibition measured. Similarly, Figure B-7 and Figure B-8 display results for electrodes 3 & 4 and 5 & 6, respectively. Table B-10 provides summary information for four electrodes, 3 through 6.

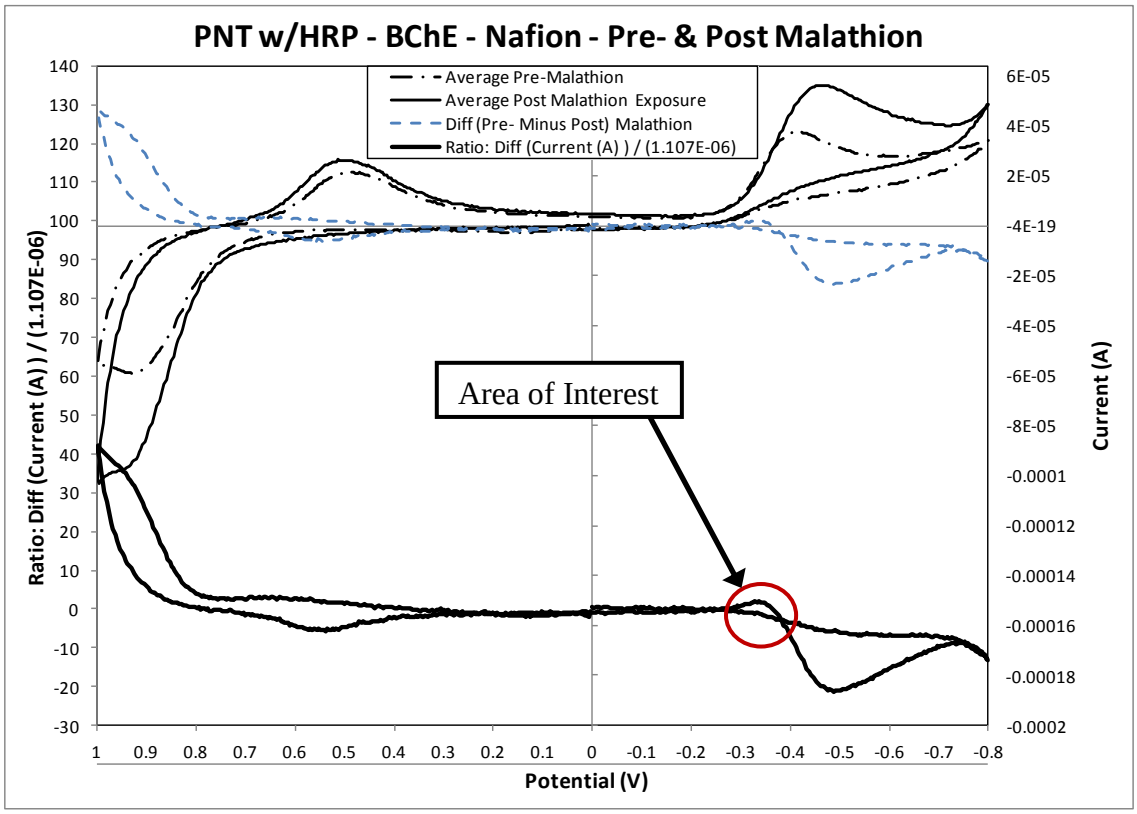

Figure B-6: PNT – HRP, BChE and Nafion Test

Table B-9: BChE Inhibition Peak Using Screen Printed Electrode

Test Configuration One		
Electrode #	**Potential (V)**	**Inhibition (%)**
1	-0.30 through -0.31	39.41
2	-0.31 through -0.34	41.11
Result Average	**-0.30 through -0.32**	**40.18**

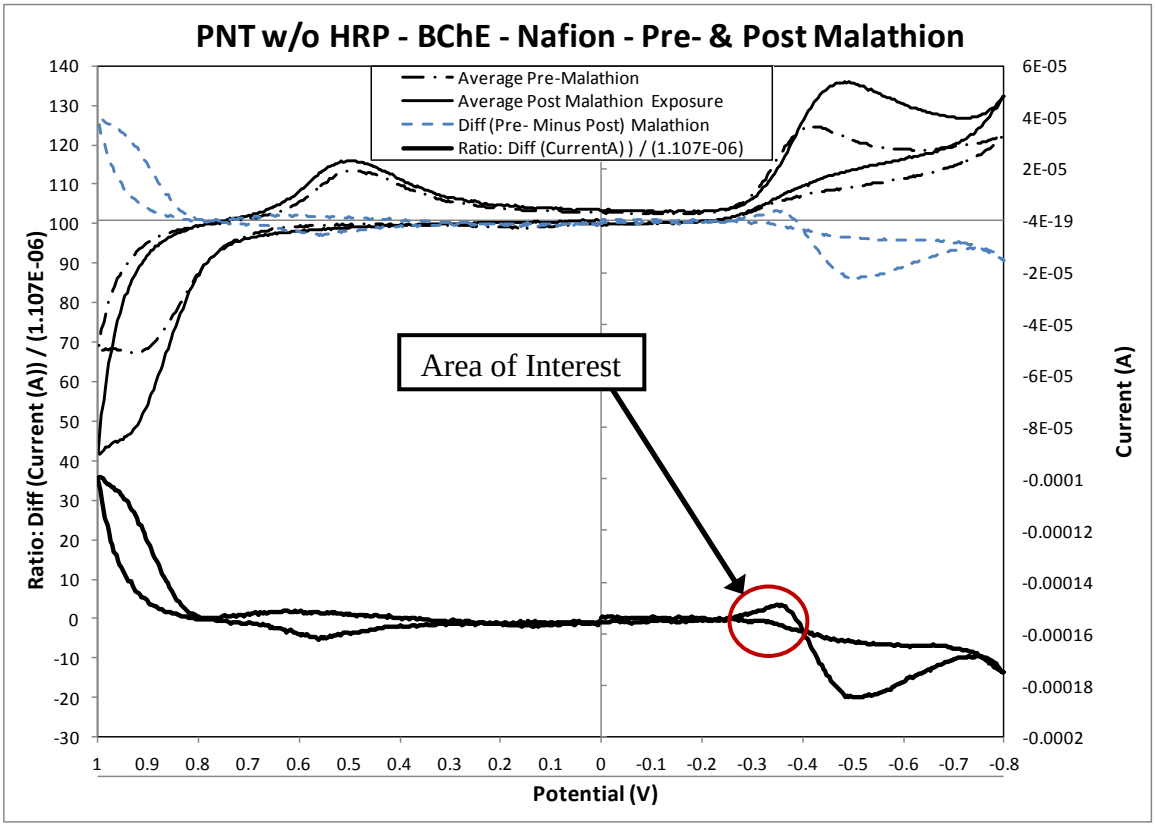

Figure B-7: PNT without HRP, BChE, and Nafion Test

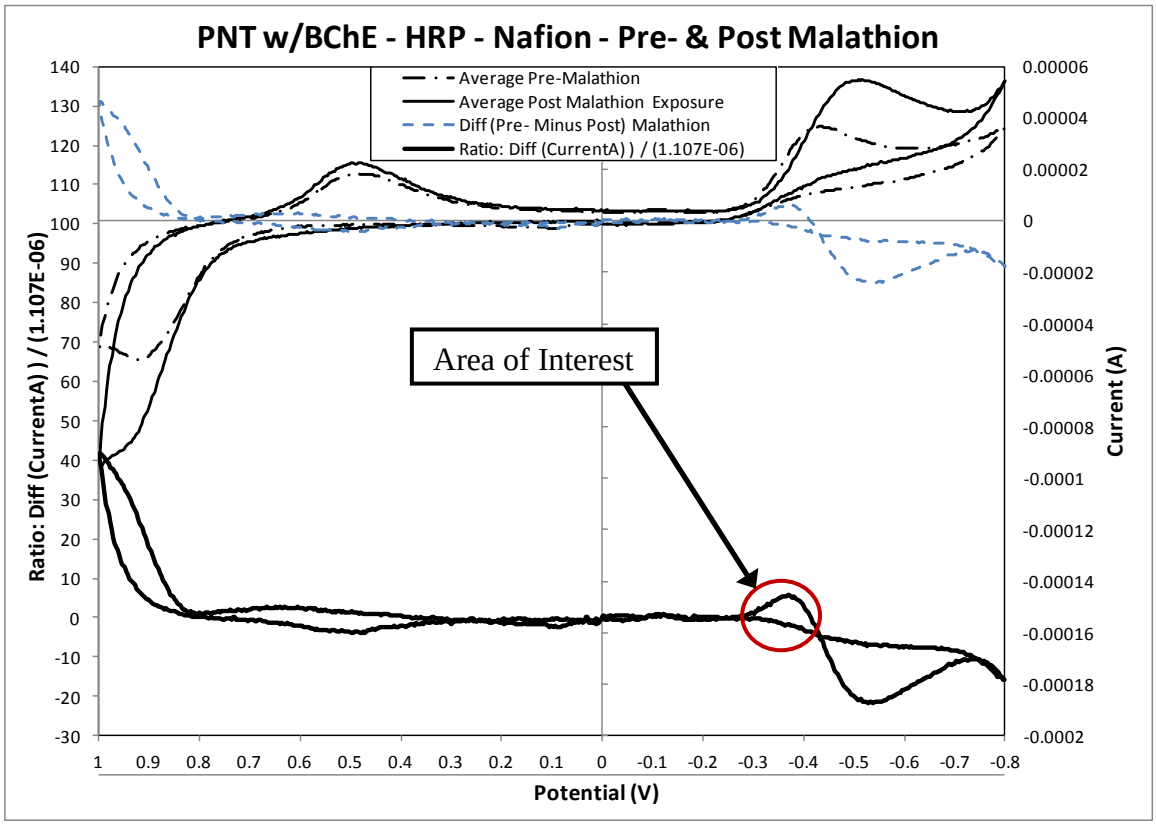

Figure B-8: PNT with BSCE, HRP, and Nafion Test

Table B-10: BChE without HRP and BChE Encapsulation Summary

Test Configuration Two		
Electrode #	**Potential (V)**	**Inhibition (%)**
3	-0.29 through -0.30	39.44
4	-0.30 through -0.34	51.51
Result Average	**-0.30 through -0.34**	**45.28**
Test Configuration Three		
Electrode #	**Potential (V)**	**Inhibition (%)**
5	-0.33 through -0.34	39.41
6	-0.34 through -0.35	57.18
Result Average	**-0.34**	**50.95**

In order to better illustrate and compare the standardized CV curves in Figures B-6 through B-8 another graph was developed with all three onto Figure B-9. Based on this information, the recipe formulation with the greatest Inhibition demonstration was PNT with BSCE encapsulated followed by HRP application and Nafion added as a protective cover.

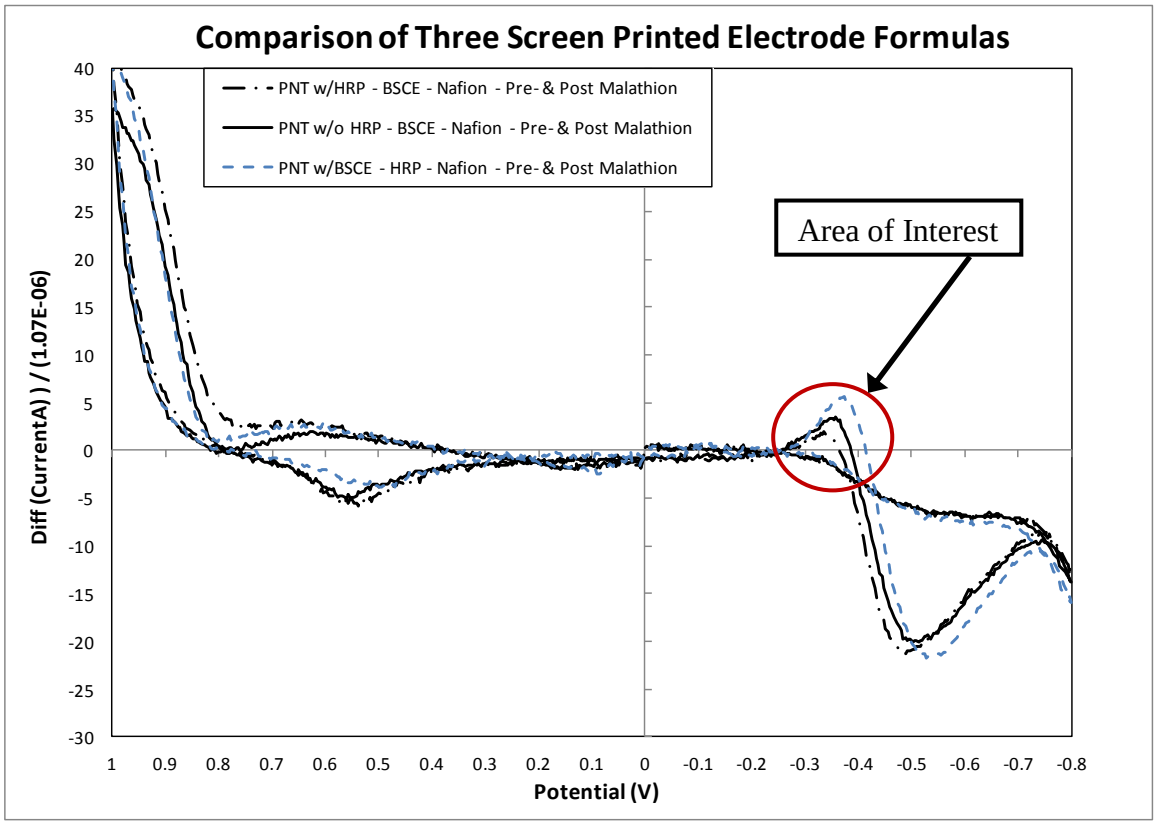

Figure B-9: Comparison of Three Sensor Formulas

B-6 PNT w/HRP Experiment:

An initial experiment performed on 16 Aug 13, failed to produce results consistent with the literature review. Specifically, the PNT encapsulation of HRP failed to enhance the CV curve and the corresponding expected measurement of chemical detection through inhibition. Upon review, the 16 Aug TCO formula utilized a PNT w/HRP solution nearly two months old. If the HRP was no longer chemically active, this would explain the 16 Aug TCO results. To test this hypothesis, a new batch of PNTs w/HRP was created to retest the 16 Aug TCO. See Section B-6, Verifying 16 Aug Test Configuration One (TCO) Test Result, for more details. Retesting the 16 Aug TCO on 9 Sep resulted in a different CV response curve. For malathion detection, HRP has a significant, enhancing impact. Based on the 9 Sep TCO validation test, the 16 Aug TCO data is rejected due to apparent chemical inactivity of the HRP solution.

Verifying 16 Aug Test Configuration One (TCO) Test Result:

On 5 Sep and 29 Oct, three electrodes were prepared with 2.5 µL of PNTs encapsulating HRP. All three electrodes had 2.5 µL BChE applied to the working electrode surface and allowed to dry. The electrodes were coated with Nafion, allowed to dry, and then were tested. See Table B-11 for a synopsis of the biosensor development and test protocol. The electrode was immersed in PBS and a cyclic voltammeter, CV#1, was taken. Afterward, the electrode was removed from the solution and immersed in a PBS with one millimolar BChE solution and a CV was again taken. The electrode was inserted into a gaseous environment for two minutes of exposure containing 25 ppbv malathion gas and another cyclic voltammogram was taken. Finally, the electrode was reintroduced to the BSCh solution and another CV test was administered.

Table B-11: BChE - Reusable Biosensor Preparation:

Surface Preparation:	Six electrodes prepared with acetate cleaning solution. HRP encapsulated PNTs applied to surface and allowed to dry. BChE mid-layer added to three electrodes. Nafion dissolved in DI water applied as top-cover. After each application, electrodes were dried using nitrogen.
Biosensor Conditioning:	Initial immersion into phosphate buffer solution. Cyclic voltammeter (CV #1) test applied. Immersed in ATCh solution and CV #2 applied.
Gas Phase Exposure:	Inserted into 25 ppbv Malathion gas vapor for two minutes. CV #3 applied.
Inhibition Measurement:	Immediately re-inserted into ATCh solution Applied CV #4 test.

Analysis of the CV signatures for BSCh are notably different between pre- and post malathion exposure. To better understand the difference, a third line was developed and plotted in Figure B-10 that subtracted the BSCh pre-malathion exposure from the post malathion exposure. Finally, the "Difference" calculation was divided by the original corresponding BChE data points to produce a standardized ratio. From this, the distinctive

region of potential interest was again noticed. BChE with Nafion has a distinctive CV fingerprint region at about -0.30 through -0.40 volts. After reviewing the area of potential interest, Table B-12 & B-13 records the percentage of malathion inhibition measured on 5 Sep and 29 Oct, respectively. Both individual electrode information as well as the summary curve was recorded.

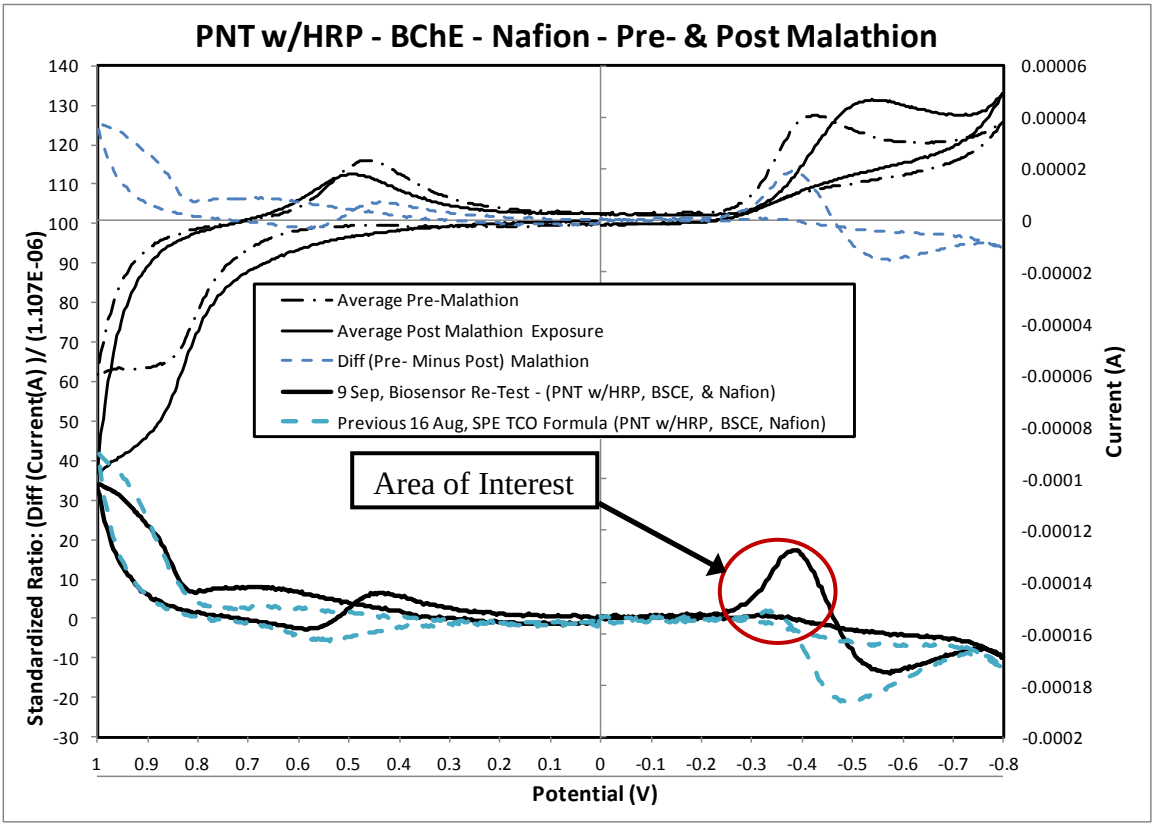

Figure B-10: PNT w/HRP - BChE/BSCh Nafion Biosensor Re-Test (5 Sep)

Table B-12: BChE Inhibition Peak Using PNT w/HRP & Nafion - 5 Sep

Electrode #	Potential (V)	Inhibition (%)
4	-0.34 through -0.36	70.37
5	-0.33 through -0.34	69.18
6	-0.34 through -0.34	68.29
Peak Average **Std Dev**		**69.28** **1.04**
Result Average	**-0.34 through -0.34**	**67.58**

Table B-13: BChE Inhibition Peak Using PNT w/HRP & Nafion – 29 Oct

Electrode #	Potential (V)	Inhibition (%)
4	-0.32 through -0.35	68.16
5	-0.32 through -0.35	65.74
6	-0.35 through -0.36	63.13
Peak Average **Std Dev**		**65.68** **2.52**
Result Average	**-0.32 through -0.35**	**65.48**

B-7 Conducting Electrode Experiment Without Nafion:

One set of electrodes was prepared with 2.5 µL of PNTs encapsulating HRP. Just prior to testing, 2.5 µL of BChE was added on top of the PNTs. Once dry, the electrode was immersed in PBS and a CV was taken. Then the electrode was removed from the solution and immersed in a PBS with one millimolar BSCh and CV#2 was again taken. The electrode was inserted into a gaseous environment containing malathion gas for two minutes and CV#3 was applied. Finally, the electrode was re-inserted into the BSCh solution to measure the post malathion inhibition. The experiment protocol is summarized in the following table.

Table B-14: Sensor Experiment without Nafion Protective Cover:

Surface Preparation:	Six electrodes prepared with acetate cleaning solution. HRP encapsulated PNTs applied to surface and allowed to dry. BChE mid-layer added to three electrodes. After each application, electrodes were dried using nitrogen.
Biosensor Conditioning:	Initial immersion into phosphate buffer solution. Cyclic voltammeter (CV #1) test applied. Immersed in BSCh solution and CV #2 applied.
Gas Phase Exposure:	Inserted into 25 ppbv Malathion gas vapor for two minutes. CV #3 applied.
Inhibition Measurement:	Immediately re-inserted into BSCh solution. Applied CV #4 test.

Analysis of the CV signatures for BSCh is notably different when Nafion is absent. To better understand the difference, a third line was developed and plotted in Figure B-11 that subtracted the BSCh pre-malathion exposure from the post malathion exposure. Finally, the "Difference" calculation was divided by the original corresponding BChE data points to produce a standardized ratio. From this, the distinctive region of potential interest was again noticed. BChE without Nafion has a distinctive CV fingerprint region at about -0.30 - -0.40 volts. After reviewing the area of potential interest, Table B-15 records the percentage of malathion inhibition measured. Both individual electrode information as well as the summary curve was recorded.

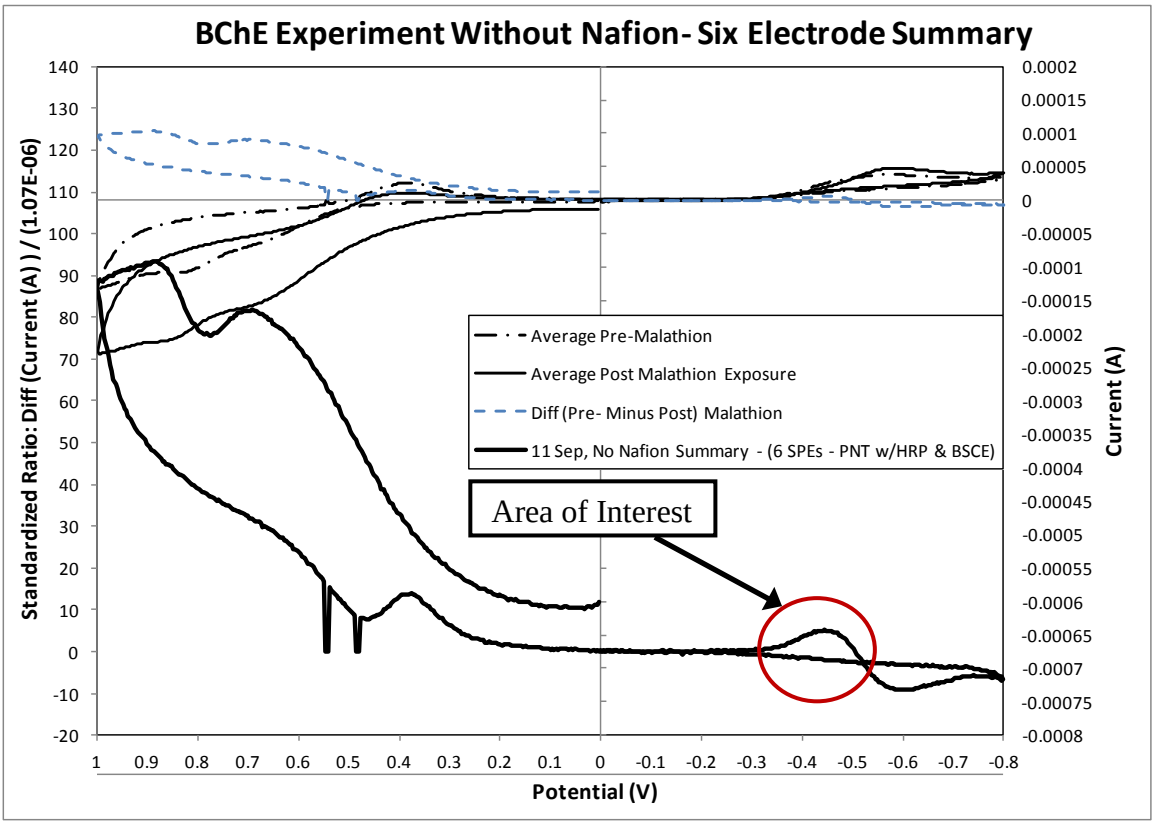

Figure B-11: BChE/BSCh Biosensor without Nafion

Table B-15: BChE Inhibition Peak Using PNT w/HRP & Nafion

Electrode #	Potential (V)	Inhibition (%)
1	-0.39 through -0.40	52.61
2	-0.39 through -0.42	32.39
3	-0.40 through -0.41	38.04
4	-0.42 through -0.43	26.43
5	-0.42 through -0.43	20.67
6[1]	-0.46[1]	4.06[1]
Average **Std Dev**		**34.03** **12.25**
Result Average	**-0.42 through -0.43**	**27.09**
Note 1: Results from this electrode were non-consistent and unusable.		

B-8 Sensitivity Experiment With Fifty Percent Concentration – 12.5 ppbv:

On 27 Sep 13, a set of electrodes was prepared according to Table B-16 for the purpose of determining the biosensor sensitivity. Six 40ml vials were also prepared the previous day using 20 ml of malathion vapor gas at 25 ppbv combined with 20 ml nitrogen gas using a laboratory method developed by Peter Baker in his research.

Table B-16: Sensor Experiment with Malathion at 12.5 ppbv:

Surface Preparation:	Six electrodes prepared with acetate cleaning solution. HRP encapsulated PNTs applied to surface and allowed to dry. BChE mid-layer added to three electrodes. After each application, electrodes were dried using nitrogen.
Biosensor Conditioning:	Initial immersion into phosphate buffer solution. Cyclic voltammeter (CV #1) test applied. Immersed in BSCh solution and CV #2 applied.
Gas Phase Exposure:	Inserted into 25 ppbv Malathion gas vapor for two minutes. CV #3 applied.
Inhibition Measurement:	Immediately re-inserted into BSCh solution. Applied CV #4 test.

Analysis of the CV signatures for BSCh indicates lower detection through inhibition. To better understand the difference, a third line was developed and plotted in Figure B-12 that subtracted the BSCh pre-malathion exposure from the post malathion exposure. Finally, the "Difference" calculation was divided by the original corresponding BChE data points to produce a standardized ratio. From this, the distinctive region of potential interest was again noticed. BChE detection at fifty percent concentration has a distinctive CV fingerprint region at about -0.30 through -0.40 volts. After reviewing the area of potential interest, Table B-17 records the percentage of malathion inhibition measured. Both individual electrode information as well as the summary curve was recorded.

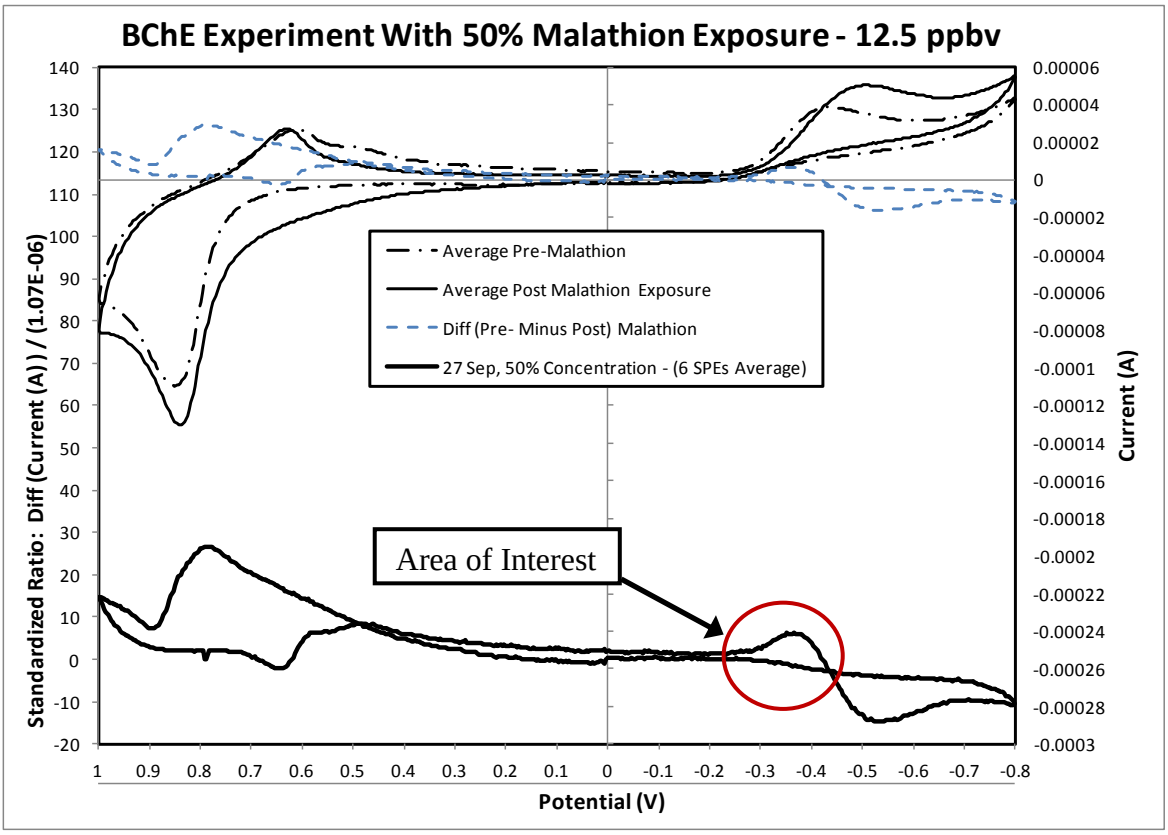

Figure B-12: BChE/BSCh Biosensor with Fifty Percent Malathion

Table B-17: BChE Inhibition Peak with Fifty Percent Malathion

Electrode #	Potential (V)	Inhibition (%)
1	-0.33 through -0.34	33.46
2	-0.32 through -0.34	36.72
3	-0.31 through -0.32	50.63
4	-0.33 through -0.35	40.50
5	-0.34 through -0.36	30.47
6	-0.35 through -0.36	55.79
Peak Average **Std Dev**		**41.26** **9.97**
Result Average	**-0.32 through -0.34**	**40.27**

Testing the BChE sensor with fifty percent malathion exposure produces a diminished characteristic peak in the potential area of interest. Experimental results for the peak amount of Inhibition is more variable; based on previous data, BChE biosensors can detect malathion at 12.5 ppbv. The 27 Sep exposure test affirms that with the right quality control and experimental set up malathion can be sensed at 12.5 ppbv.

B-9 Sensitivity Experiments with Sixty, Seventy-five, and Eighty Percent Concentration:

On 2 Oct, 30 Sep, and 3 Oct 13 a set of electrodes was prepared according to Table B-18 for the purpose of determining the sensitivity at sixty, seventy-five and eighty percent concentration, respectively. Six 40 ml vials were prepared the day prior day using malathion vapor gas at 25 ppbv combined with nitrogen gas to achieve the appropriate gas vapor concentration.

Table B-18: Experiments with Malathion at Varied Concentration:

Surface Preparation:	Six electrodes prepared with acetate cleaning solution. HRP encapsulated PNTs applied to surface and allowed to dry. BChE mid-layer added to three electrodes. After each application, electrodes were dried using nitrogen.
Biosensor Conditioning:	Initial immersion into phosphate buffer solution. Cyclic voltammeter (CV #1) test applied. Immersed in BSCh solution and CV #2 applied.
Gas Phase Exposure:	Inserted into 25 ppbv Malathion gas vapor for two minutes. CV #3 applied.
Inhibition Measurement:	Immediately re-inserted into BSCh solution. Applied CV #4 test.

Analysis of the CV signatures for BSCh indicates inhibition. To better understand malathion interaction, a third line was developed and plotted in Figure B-13, B-14, and B-15 showing the difference between the BSCh pre-malathion exposure and the post malathion exposure for the varied concentrations. From this, the region of potential interest was noticed. BChE detection at seventy-five percent concentration has a distinctive CV fingerprint region at about -0.30 through -0.40 volts. During the 75% malathion exposure experiment, electrodes four and five appeared to show almost no CV inhibition response curve and was likely due to no malathion exposure. See figures B-16 and B-17 for additional detail. Review of the method used to make the 75% malathion concentration for electrode 6 indicated it had less than 5 ppbv malathion. The malathion vials for electrodes 4 through 6 appear to have had less than 18.75 ppbv malathion concentration. For the seventy five percent concentration experiment, electrodes 4 through 6 were excluded from the summary data. Tables B-19, B-20, and B-21 summarize the malathion inhibition.

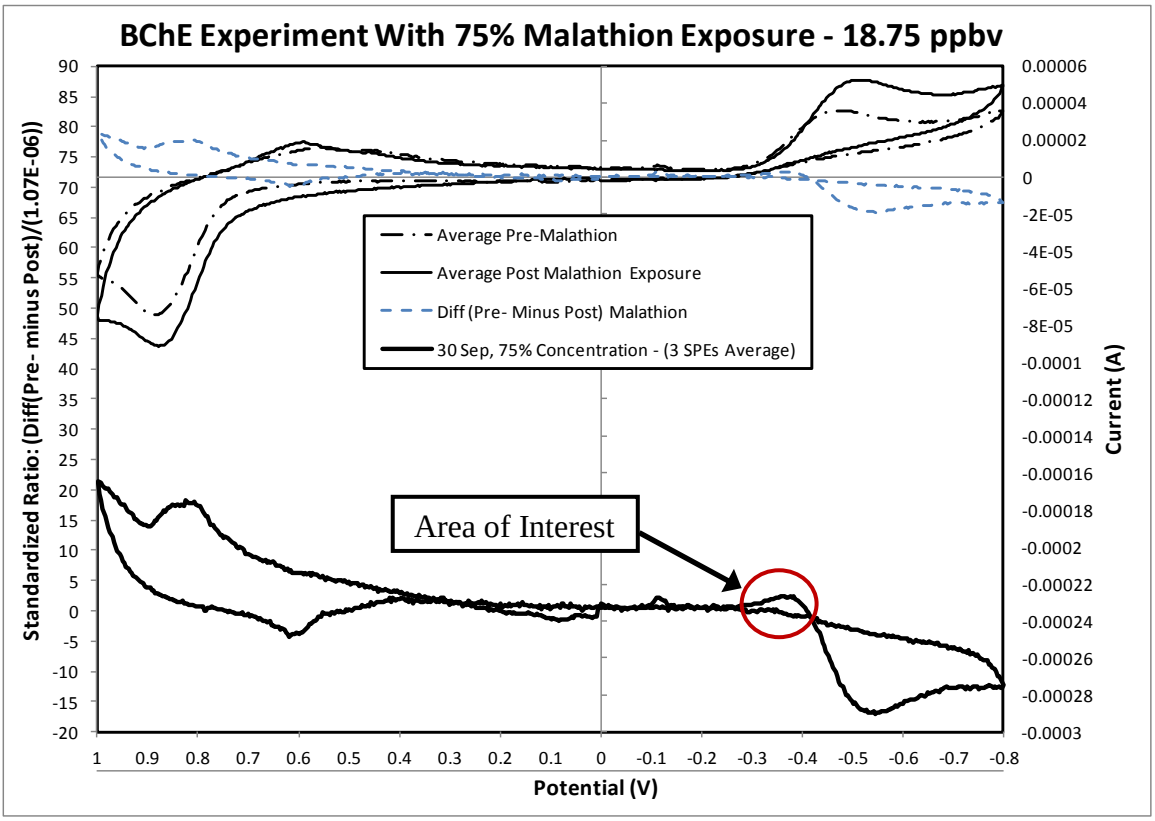

Figure B-13: BChE/BSCh Biosensor with Seventy Five Percent Malathion Exposure

Table B-19: BChE Inhibition Peak with 75% Malathion

Electrode #	Potential (V)	Inhibition (%)
1	-0.32 through -0.33	44.22
2	-0.34 through -0.35	39.32
3	-0.31 through -0.32	33.28
4	-0.33 through -0.35	-1.47[1]
5	-0.32 through -0.35	6.86[1]
6	-0.32 through -0.34	27.87[2]
Peak Average **Std Dev**		**38.94**[3] **5.48**[3]
Result Average	**-0.33 through -0.33**	**37.16**[3]

Note 1: Electrodes 4 and 5 indicate non-consistent CV response, inhibition unusable.

Note 2: The gas phase concentration preparation process, electrode 6 was exposed to 4.69 ppbv malathion. (25ppbv*(0.25) * (0.75)) = 4.69 ppbv.

Note 3: Peak Average, Standard Deviation, and Result Average are based on electrodes 1 through 3 data.

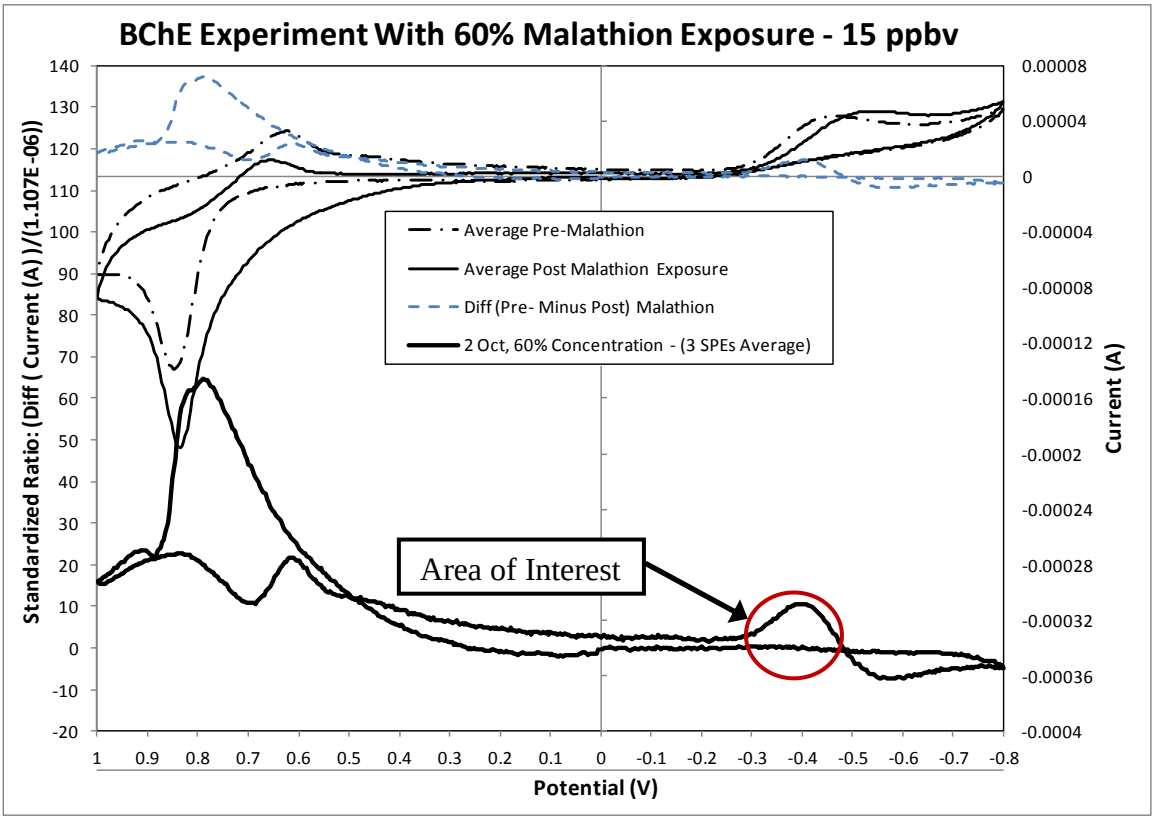

Figure B-14: BChE/BSCh Biosensor with Sixty Percent Malathion Exposure

Table B-20: BChE Inhibition Peak with Sixty Percent Malathion

Electrode #	Potential (V)	Inhibition (%)
1	-0.31 through -0.33	36.80
2	-0.32 through -0.34	13.68[1]
3	-0.32 through -0.33	41.34
4	-0.33 through -0.35	44.75
5	-	-[2]
6	-0.32 through -0.34	21.59[1]
Peak Average **Std Dev**		**40.96** **3.99**
Result Average	**-0.33 through -0.34**	**40.30**

Note 1: Exposure concentration standard for electrode 2 and 6 was six ppbv; calculated during preparation as {25 ppbv * (16/40) * (24/40)}

Note 2: CV curve results for electrode 5 were non-consistent and unusable.

Electrodes 2, 5 and 6 were excluded from 60% malathion summary data.

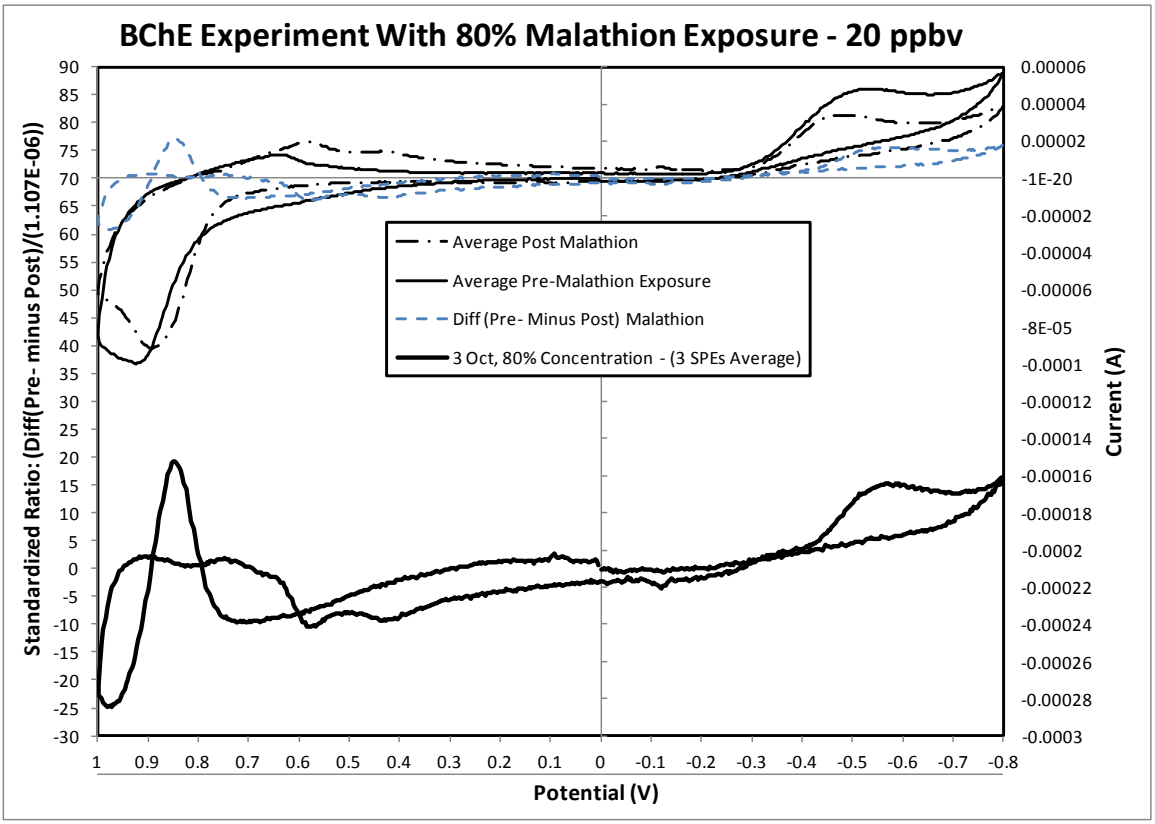

Figure B-15: BChE/BSCh Biosensor with Eighty Percent Malathion Exposure

Table B-21: BChE Inhibition Peak with 80% Malathion

Electrode #	Potential (V)	Inhibition (%)
1	-0.35 through -0.36	70.78[1]
2	-0.49 through -0.50	51.42
3	-0.49 through -0.49	42.60
4	-0.38 through -0.39	52.79
5	-0.36 through -0.37	28.74[2]
6	-0.31 through -0.32	30.72[2]
Peak Average **Std Dev**		**48.94**[3] **5.53**[3]
Result Average	**-0.49 through -0.50**	**44.57**

Note 1: Electrode 1 used a malathion sample at 100% concentration, 25 ppbv.
Note 2: Electrode 5 and 6 were run with vials containing 5 ppbv malathion concentration, 25ppbv * (8/40) = 5 ppbv.
Note 3: Electrodes 1, 5, and 6 were excluded from summary data calculations.

Testing the BChE sensor with sixty, seventy five, and eighty percent malathion exposure produces a consistent, diminished characteristic peak in the potential area of interest. These multiple exposure tests affirm malathion inhibition can be measured at 20, 18.75 and 15 ppbv.

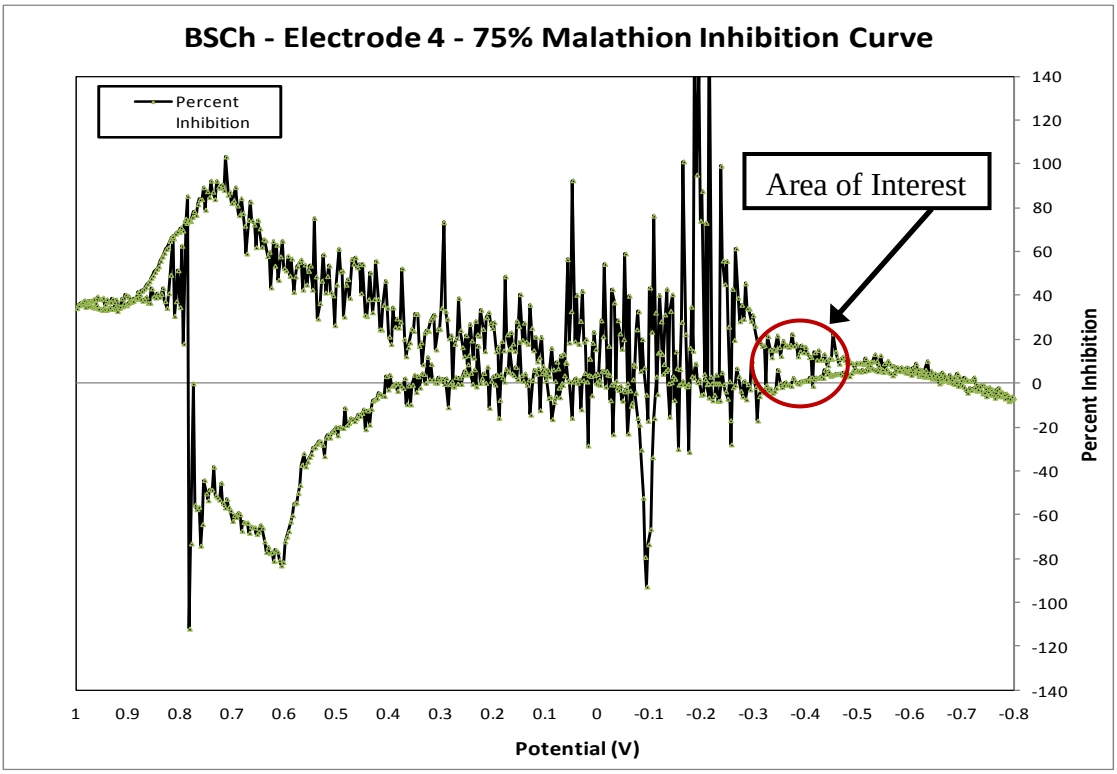

Figure B-16: CV Curve Indicating Minimal Inhibition during Experiment

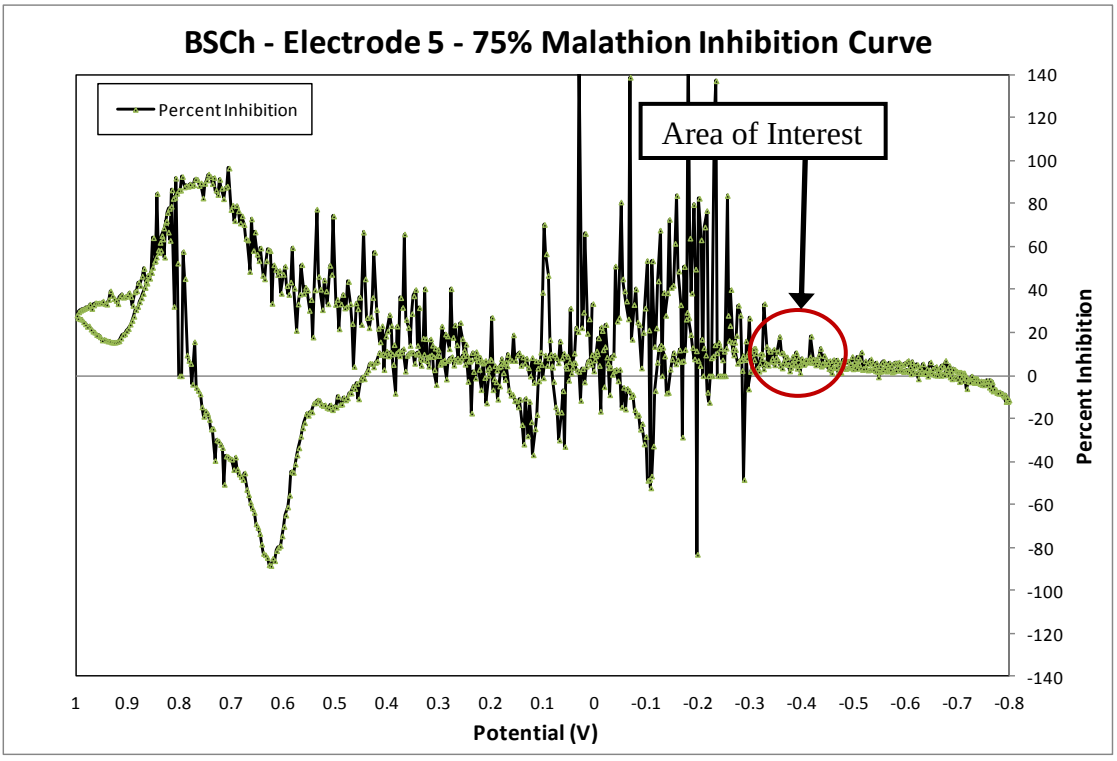

Figure B-17: CV Curve Indicating No Inhibition during Experiment

B-10 Sensitivity Experiments with Forty and Twenty Five Percent Concentration:

On 4 Oct, two sets of electrodes was prepared according to Table B-22 for the purpose of determining the sensitivity at forty and twenty five percent concentration, respectively. Twelve 40ml vials were prepared the day prior day using malathion vapor gas at 25 ppbv combined with nitrogen gas to achieve the appropriate gas vapor concentration for each experiment.

Table B-22: Experiments with Malathion at Varied Concentration:

Surface Preparation:	Six electrodes prepared with cleaning solution. HRP encapsulated PNTs applied to surface and allowed to dry. BChE mid-layer added to three electrodes. After each application, electrodes were dried using nitrogen.
Biosensor Conditioning:	Initial immersion into phosphate buffer solution. Cyclic voltammeter (CV #1) test applied. Immersed in BSCh solution and CV #2 applied.
Gas Phase Exposure:	Inserted into 25 ppbv Malathion gas vapor for two minutes. CV #3 applied.
Inhibition Measurement:	Immediately re-inserted into BSCh solution. Applied CV #4 test.

Analysis of the CV signatures for BSCh indicate inhibition. To better understand malathion interaction, refer to Figure B-18 and B-19 showing the difference between the BSCh pre-malathion exposure and the post malathion exposure. The area of interest is highlighted. BChE detection at forty percent concentration has a distinctive CV fingerprint region at about -0.30 through -0.40 volts. During SPE preparation, a new cleaning agent, potassium bromide, was utilized on SPEs two through six. During the twenty five percent malathion exposure experiment, electrodes two, three, and four indicated a right shifted Potential (V) peak; it was likely due to potassium bromide, KBr, cleaning agent residual. See Figures B-19 through B-21 for additional detail. For the twenty five percent concentration experiment, SPEs 5 and 6 were excluded from the summary data. Tables B-23 and B-24 summarize the malathion inhibition.

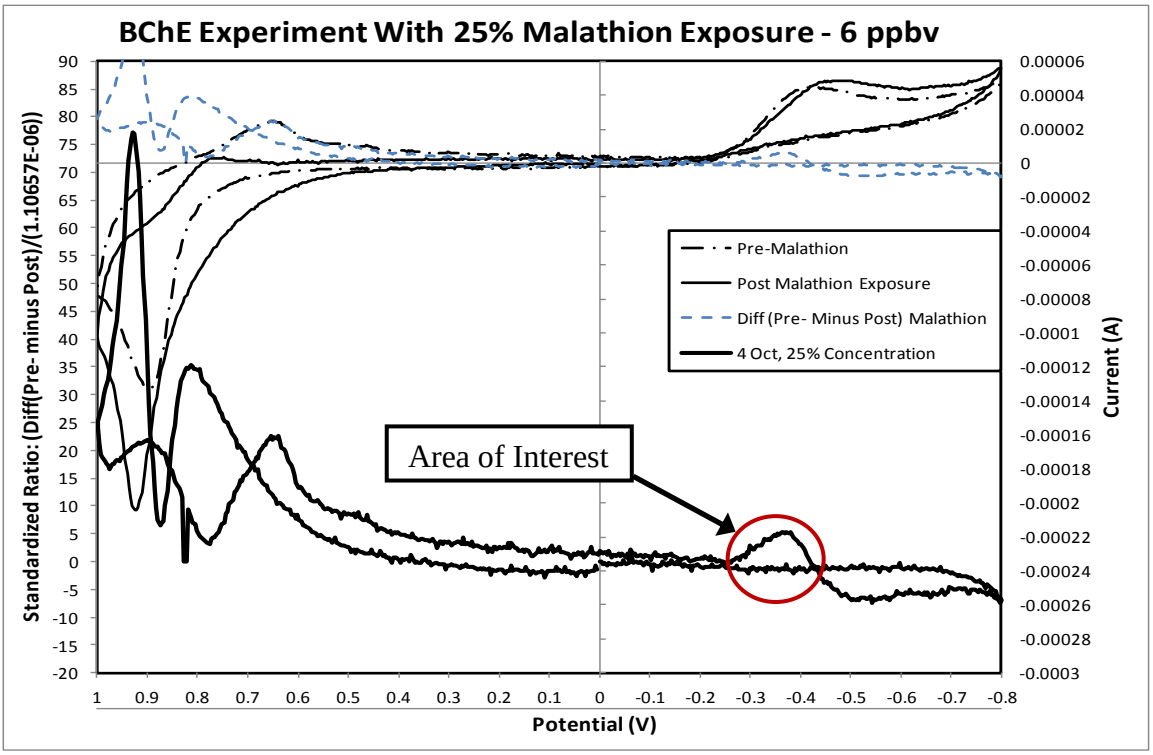

Figure B-18: BChE/BSCh Biosensor with Twenty-Five Percent Malathion

Table B-23: BChE Inhibition Peak with 25% Malathion

Electrode #	Potential (V)	Inhibition (%)
1	-0.33 through -0.37	15.91
2	-0.46 through -0.47	12.95[1]
3	-0.45 through -0.46	12.45[1]
4	-0.44 through -0.46	12.40[1]
5	-[2]	-[2]
6	-[2]	-[2]
Peak Average **Std Dev**		**13.43**[3] **1.67**[3]
Result Average	**-0.39 through -0.41**	**12.35**[3]

Note 1: During the electrode preparation process, electrodes 2, 3, and 4 were cleaned using a 1mmol potassium bromide solution in lieu of acetic acid. Although the CV response curves were stable, the Potential (V) peak was right shifted.

Note 2: CV response curves for electrodes 5 and 6 were not stable and indicated no inhibition response.

Note 3: Peak Average, Standard Deviation, and Result Average are calculated using SPE 1, 2,3 and 4 data.

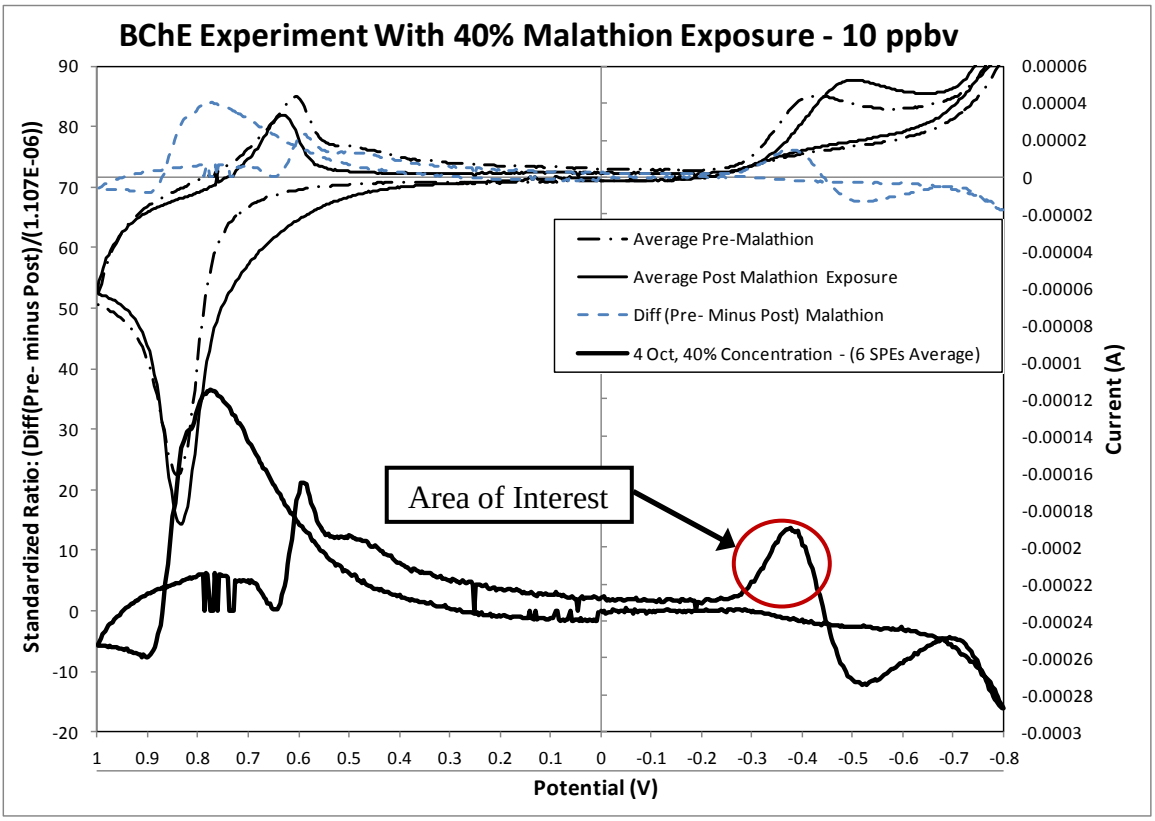

Figure B-19: BChE/BSCh Biosensor with Forty Percent Malathion Exposure

Table B-24: BChE Inhibition Peak with Forty Percent Malathion

Electrode #	Potential (V)	Inhibition (%)
1	-0.32 through -0.33	54.99
2	-0.32 through -0.33	52.71
3	-0.33 through -0.34	56.13
4	-0.34 through -0.35	48.30
5	-0.34 through -0.35	40.96[1]
6	-0.33 through -0.34	21.84[2]
Peak Average **Std Dev**		**45.82**[3] **12.98**[3]
Result Average	**-0.33 through -0.34**	**45.09**[3]

Note 1: SPE 5 utilized PNT/HRP solution prepared one month prior.
Note 2: SPE 6 utilized PNT/HRP solution prepared two months prior.
Note 3: Peak Average, Standard Deviation, and Result Average were calculated using all six SPEs.

The forty percent concentration experiment utilized three PNT/HRP formula to investigate/determine the role of HRP and perhaps boost sensor sensitivity in the process.

The first four electrodes utilized a new batch of HRP solution while electrodes 5 and 6 utilized PNT/HRP solutions that developed for previous tests. This experiment appears to indicate HRP aging has a significant role in BSChE/BSCh sensitivity. Based on this inhibition curve for malathion, detection of malathion at concentrations lower than 6 ppbv is likely achievable.

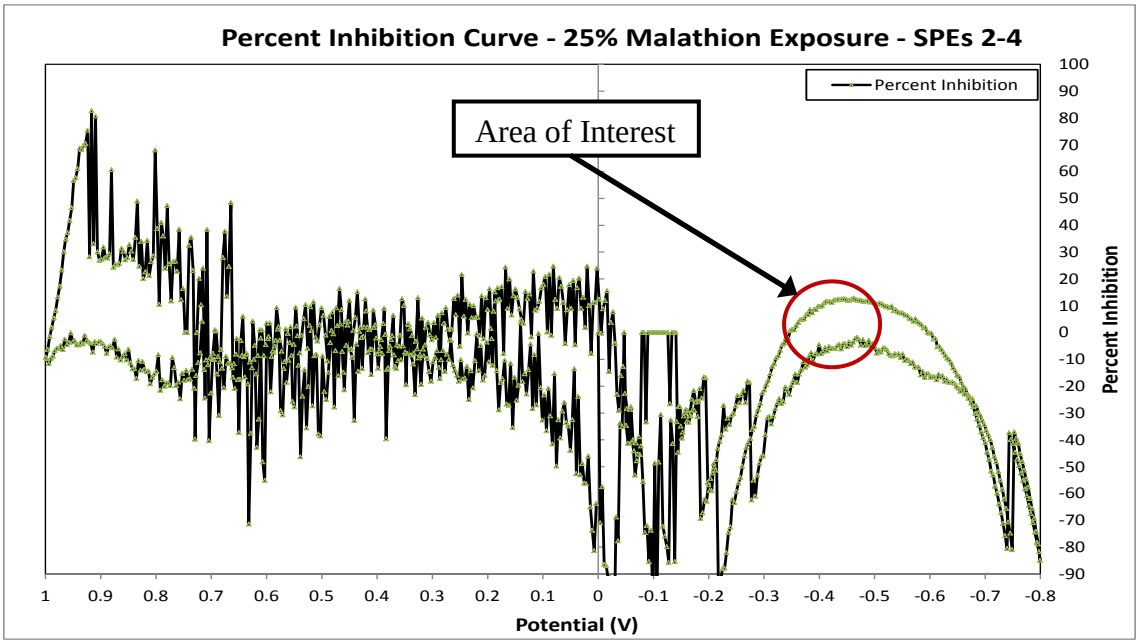

Figure B-20: Combined Inhibition Curve for Twenty Five Percent Malathion

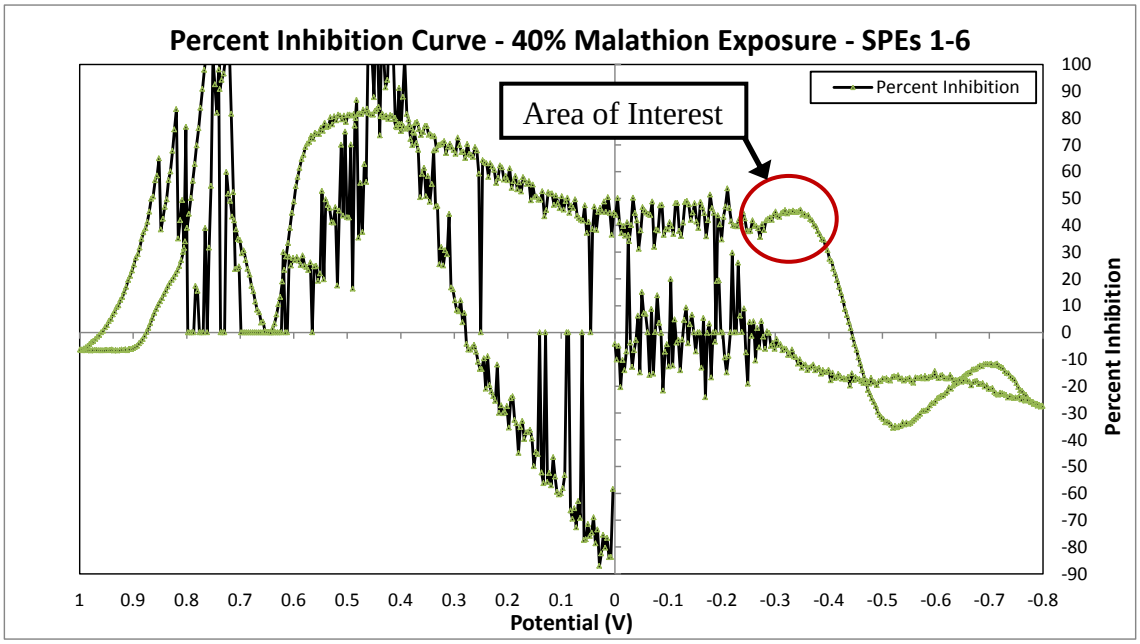

Figure B-21: CV Combined Inhibition Curve for Forty Percent Concentration

B-11 Sensitivity Experiments with Ten, Forty, and Fifty Percent Concentration:

On 15, 16, 21 Oct, and 3 Nov a set of six electrodes were prepared according to Table B-25 for the purpose of determining the sensitivity at ten, forty, fifty, and seventy-five percent concentration, respectively. Twelve 40ml vials were prepared the day prior day using malathion vapor gas at 25 ppbv combined with nitrogen gas to achieve the appropriate gas vapor concentration for each experiment.

Table B-25: Experiments with Malathion at Varied Concentration:

Surface Preparation:	Six electrodes prepared with cleaning solution. HRP encapsulated PNTs applied to surface and allowed to dry. BChE mid-layer added to three electrodes. After each application, electrodes were dried using nitrogen.
Biosensor Conditioning:	Initial immersion into phosphate buffer solution. Cyclic voltammeter (CV #1) test applied. Immersed in BSCh solution and CV #2 applied.
Gas Phase Exposure:	Inserted into 25 ppbv Malathion gas vapor for two minutes. CV #3 applied.
Inhibition Measurement:	Immediately re-inserted into BSCh solution. Applied CV #4 test.

Analysis of the CV signatures for BSCh indicate inhibition. See Figures B-22 through B-25 for additional detail. Tables B-26 through B-30 summarize malathion inhibition. The ten percent concentration experiment utilized newly created PNT/HRP formula to investigate/determine the role of HRP and perhaps boost SPE sensitivity in the process. This experiment appears to confirm HRP aging has a significant role in BSChE/BSCh sensitivity. Based on this inhibition curve test for malathion, detection of malathion at concentrations lower than 6 ppbv is achievable.

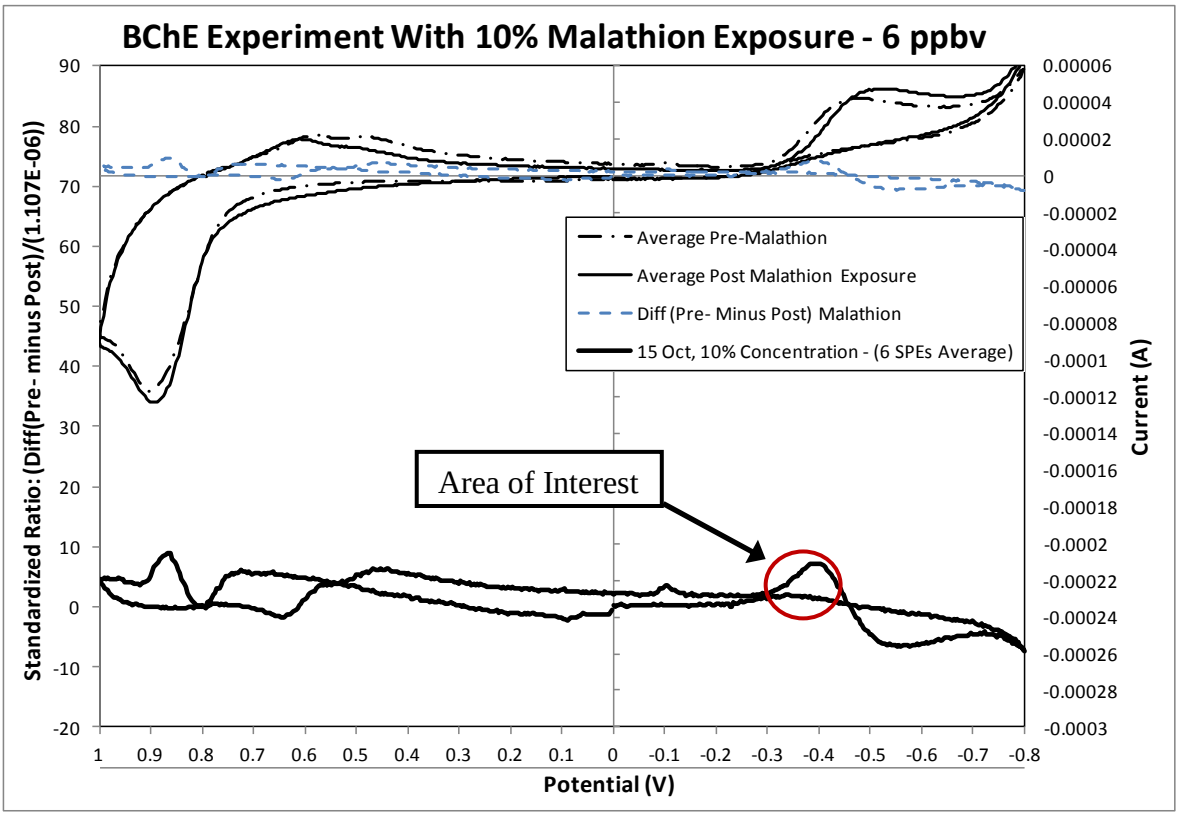

Figure B-22: BChE/BSCh Biosensor with Ten Percent Malathion Exposure

Table B-26: BChE Inhibition Peak with 10% Malathion

Electrode #	Potential (V)	Inhibition (%)
1	-0.32 through -0.35	45.93
2	-0.32 through -0.35	33.96
3	-0.32 through -0.34	40.64
4	-0.33 through -0.35	28.65
5	-0.32 through -0.33	23.44
6	-0.32 through -0.34	12.61
Average	**-0.32 through -0.33**	**31.05**
Peak Average		**30.87**
Std Dev		**12.04**

Table B-27: BChE Inhibition Peak with Forty Percent Malathion

Electrode #	Potential (V)	Inhibition (%)
1	-0.31 through -0.34	36.26
2	-0.33 through -0.34	13.95
3	-0.33 through -0.34	29.58
4	-0.32 through -0.35	21.86
5	-0.32 through -0.35	20.80
6	-0.32 through -0.34	34.36
Result Average	**-0.32 through -0.34**	**25.54**
Peak Average **Std Dev**		**26.14** **8.69**

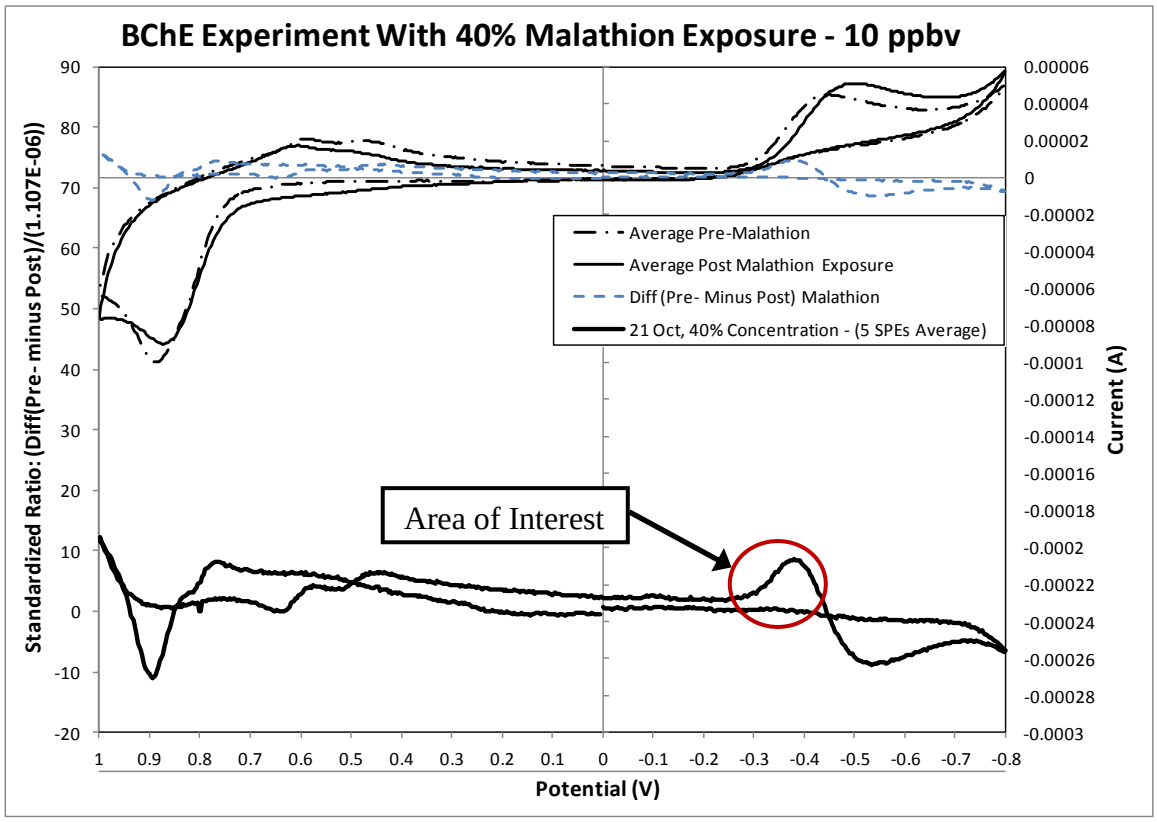

Figure B-23: BChE/BSCh Biosensor with Forty Percent Malathion Exposure

Table B-28: BChE Inhibition Peak with Forty Percent Malathion

Electrode #	Potential (V)	Inhibition (%)
1	-0.32 through -0.34	34.27
2	-0.32 through -0.33	28.77
3	-0.32 through -0.34	40.05
4	-0.33 through -0.34	29.56
5	-[1]	-[1]
6	-0.32 through -0.35	40.68
Result Average	**-0.32 through -0.34**	**34.54**
Peak Average **Std Dev**		**34.67** **5.62**
Note 1: CV response curves for this electrode was non-consistent and unusable.		

Table B-29: BChE Inhibition Peak with Fifty Percent Malathion

Electrode #	Potential (V)	Inhibition (%)
1	-0.32 through -0.33	37.63
2	-0.33 through -0.34	36.68
3	-0.32 through -0.34	33.80
4	-0.32 through -0.33	29.34
5	-0.32 through -0.35	39.90
6	-0.33 through -0.34	24.92
Result Average	**-0.32 through -0.34**	**33.50**
Peak Average **Std Dev**		**33.71** **5.64**

Table B-30: BChE Inhibition Peak with Seventy Five Percent Malathion

Electrode #	Potential (V)	Inhibition (%)
1	-0.32 through -0.35	52.01
2	-0.33 through -0.35	56.74
3	-0.32 through -0.36	58.06
4	-0.32 through -0.35	53.60
5	-0.32 through -0.35	53.86
6	-0.33 through -0.35	54.03
Result Average	**-0.33 through -0.35**	**54.42**
Peak Average **Std Dev**		**54.72** **2.24**

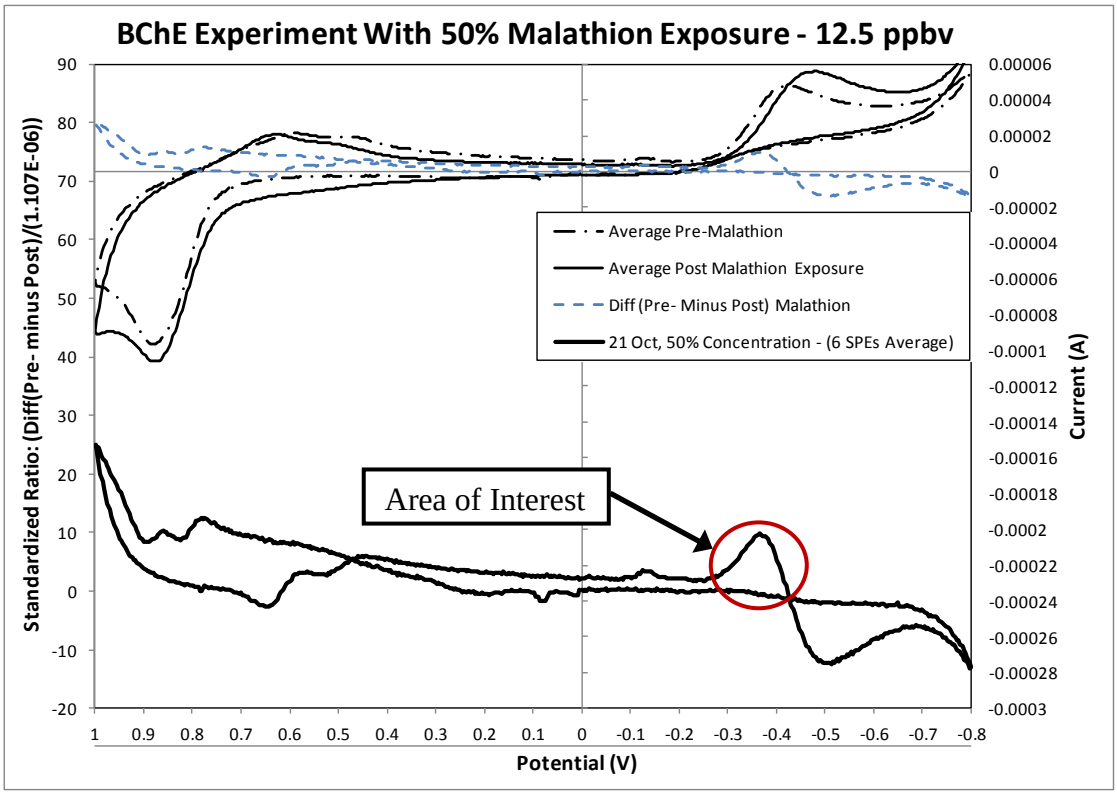

Figure B-24: BChE/BSCh Biosensor with Fifty Percent Malathion Exposure

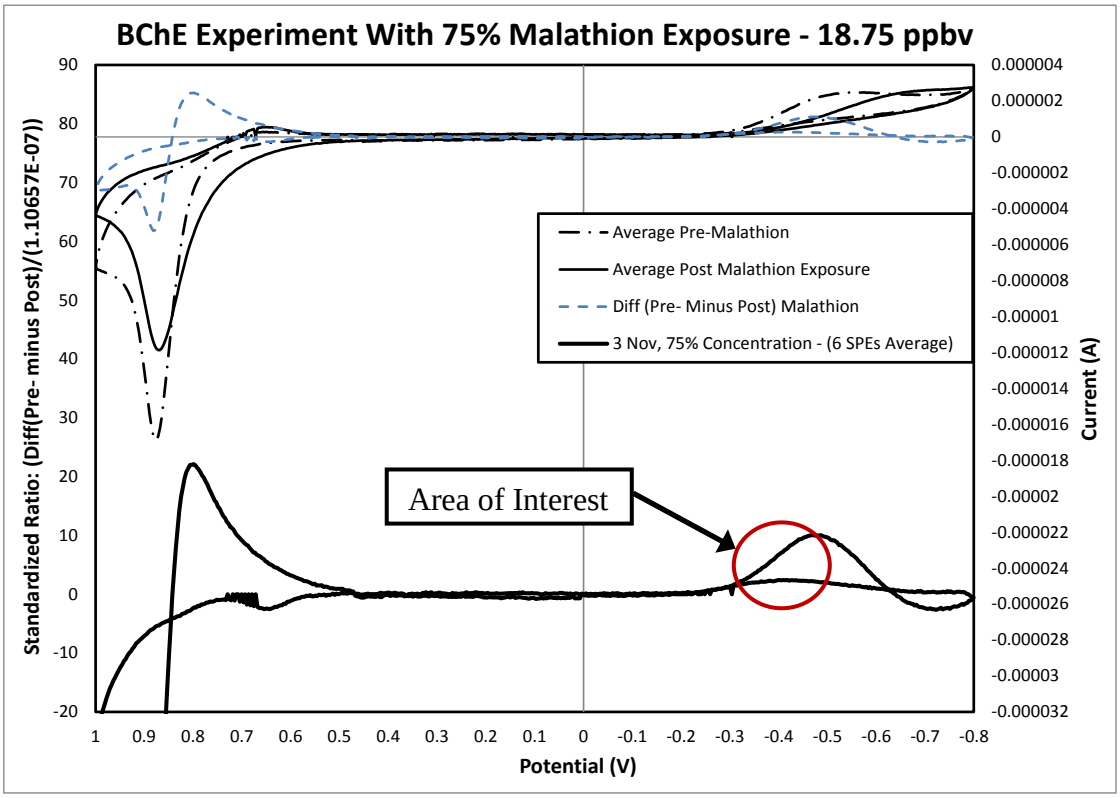

Figure B-25: BChE/BSCh Biosensor with Seventy-Five Percent Malathion Exposure

Appendix C. Longevity Exposure Experiments

Although the Week 0 experiment was conducted on 5 Sep, the electrodes for the following weeks were prepared on 3 Sep and tested every seven days thereafter. While tables C-1 and C-4 summarize the first longevity data set, tables C-5 and C-6 incorporate additional longevity test data from a second longevity test run.

Table C-1. Longevity Exposure Experiment Week 1 and 2

Electrode #	Week 1 – 10 Sep 13		Week 2 - 17 Sep13	
	Potential (V)	**Inhibition (%)**	**Potential (V)**	**Inhibition (%)**
1	-0.32 to -0.36	82.60[1]	-0.31 to -0.33	49.75
2	-0.36 to -0.37	72.37	-0.36 to -0.37	62.11
3	-0.33 to -0.34	52.41	-0.35 to -0.36	51.95
4	-0.31 to -0.32	54.05	-0.39 to -0.40	50.24
5	-0.38 to -0.39	53.65	-0.38 to -0.39	45.85
6	-0.41 to -0.42	50.68	-0.37 to -0.38	61.89
Result	**-0.34 to -0.35**	**52.95[1]**	**-0.36 to -0.37**	**50.93**
Average[2]		**56.63[1]**		**53.63**
Std Dev		**8.90**		**6.78**

Note 1: Week 1, electrode 1 was excluded from data analysis due to likely liquid malathion exposure during CV #3 test for that electrode.

Note 2: The first Result Average value is a point by point averaging of the electrode values along the CV curve. The second Result Average value is the straight value associated with the inhibition peak without respect to the potential along the CV curve.

Figures C-1 and C-2 are full CV curve plots for the longevity experiments for the first four weeks. The data lines plotted in the upper section of the graph belong to the y-axis labels indexed on the right hand side and demonstrate how the difference calculation was performed. The data lines plotted in the lower section of the graph belong to the y-axis labels on the left hand side of the graph. In order to compare the weekly results on a standardized graph, the weekly difference calculation was divided by $1.1066X10^{-06}$ so that a weekly standardized comparison could be made.

Table C-2: Longevity Exposure Experiment Week 3 and 4

Electrode #	Week 3 – 24 Sep 13		Week 4 - 1 Oct 13	
	Potential (V)	Inhibition (%)	Potential (V)	Inhibition (%)
1	-0.33 to -0.34	58.69	-0.36 to -0.37	49.36
2	-0.34 to -0.35	67.79	-0.35 to -0.35	44.34
3	-0.33 to -0.35	68.84	-0.34 to -0.35	60.81
4	-0.35 to -0.37	78.10	-0.37 to -0.38	42.12
5	-0.34 to -0.37	72.58	-0.35 to -0.37	50.15
6	-0.36 to -0.38	66.29	-0.37 to -0.38	51.64
Result	**-0.34 to -0.36**	**68.23**	**-0.35 to -0.37**	**49.18**
Average		**68.72**		**49.74**
Std Dev		**6.48**		**6.54**

Note: Week 3, BSCE/BSCh electrodes indicates higher inhibition. Although there are multiple possible reasons for improved inhibition compared to Week 1 and 2 data, the strongest likelihood is that decreasing the delay between Malathion, CV#3, exposure and CV#4 retesting of the electrode resulted in higher inhibition measurement.

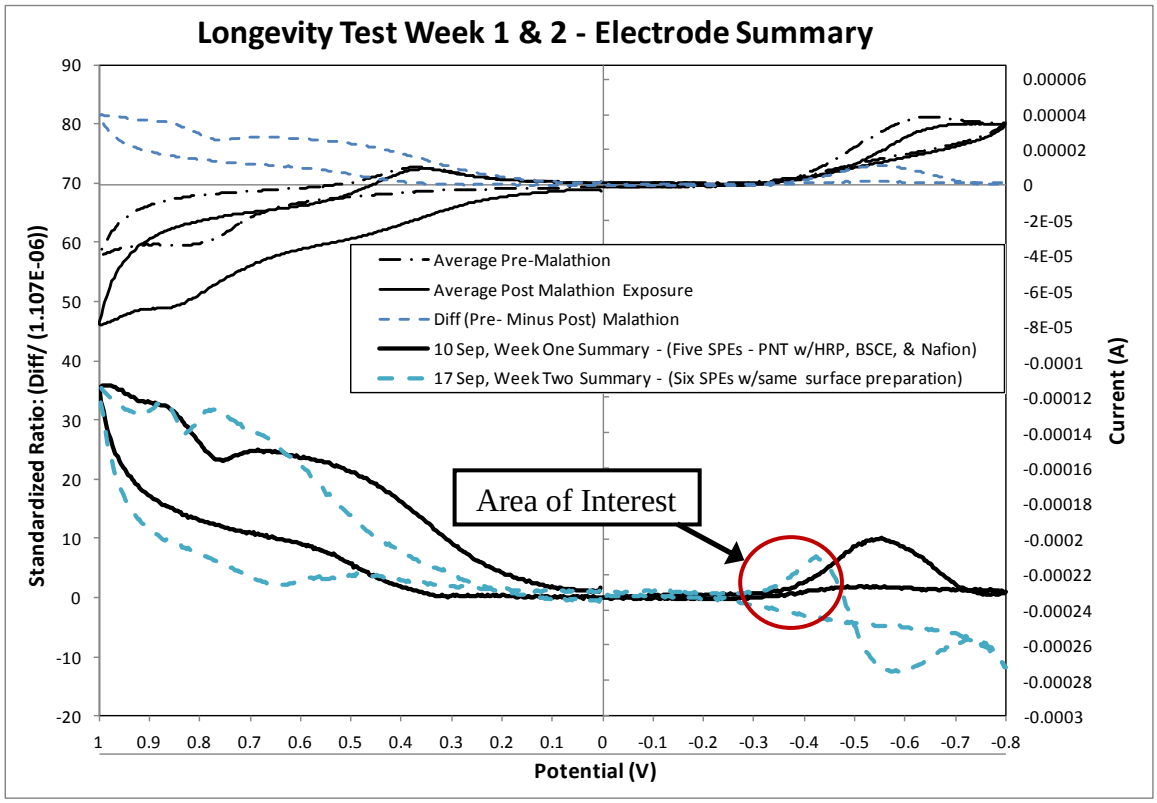

Figure C-1: Longevity Test Week One and Two

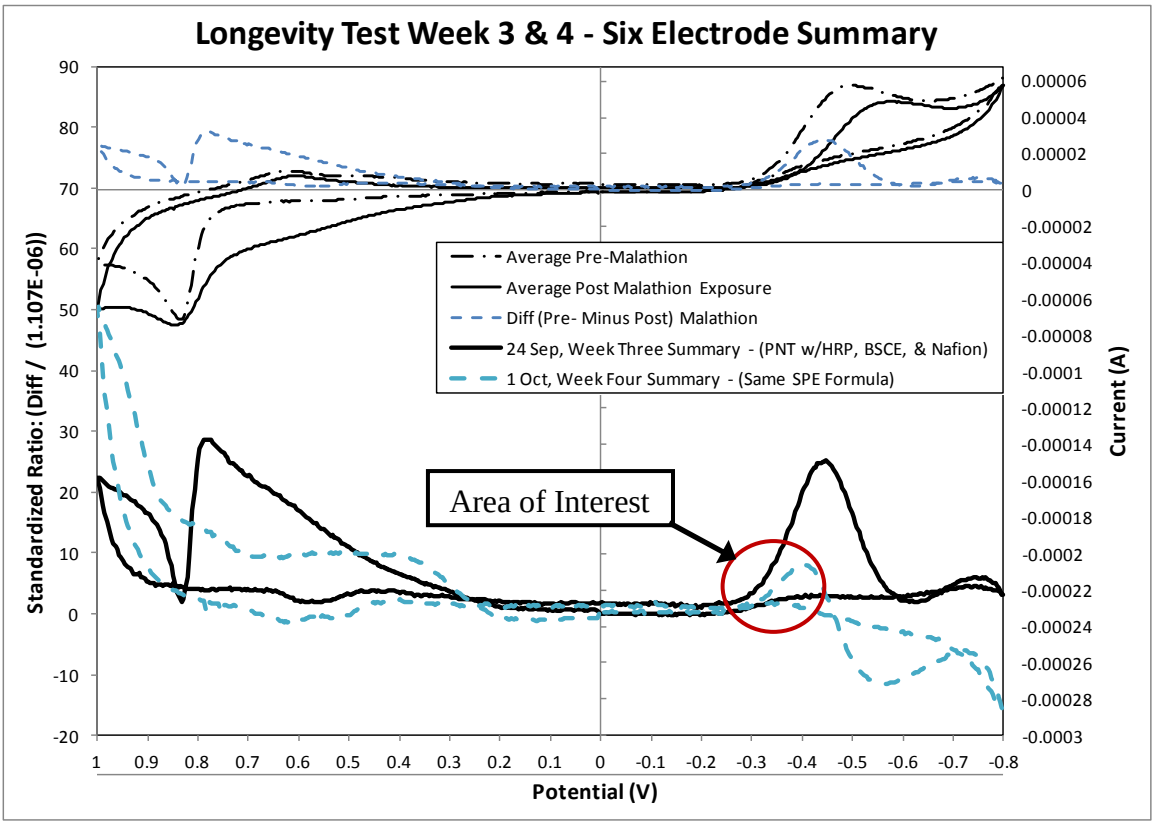

Figure C-2: Longevity Test Week Three and Four

There was one notable exception made during the comparison of individual electrode results that resulted in the exclusion of one data set. Upon data review, see Figure C-3, the CV data curve plotted during malathion exposure indicated that the electrode was either immersed in malathion or was still wet from the previous PB test and initial baseline BSCh immersion. Since this was not part of the standard protocol for gas phase detection, the data was excluded from further gas phase detection analysis.

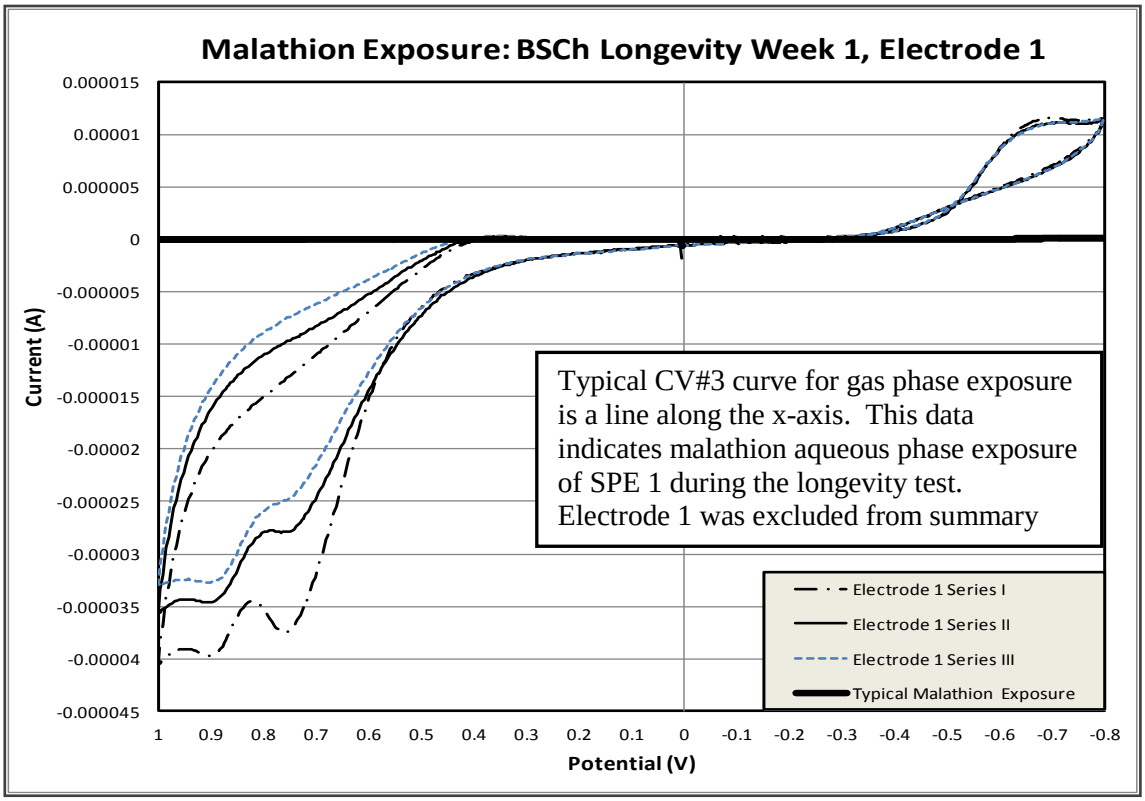

Figure C-3: Malathion Exposure: BSCh Longevity Week 1, Electrode 1 Data Anomaly

After reviewing the area of interest in Figures C-4 through C-5, tables C-3 and C-4 record the percent of malathion inhibition measured for Longevity Week's 5, 6, 8, 10, and 11. Individual electrode information was averaged to arrive at CV summary curve information.

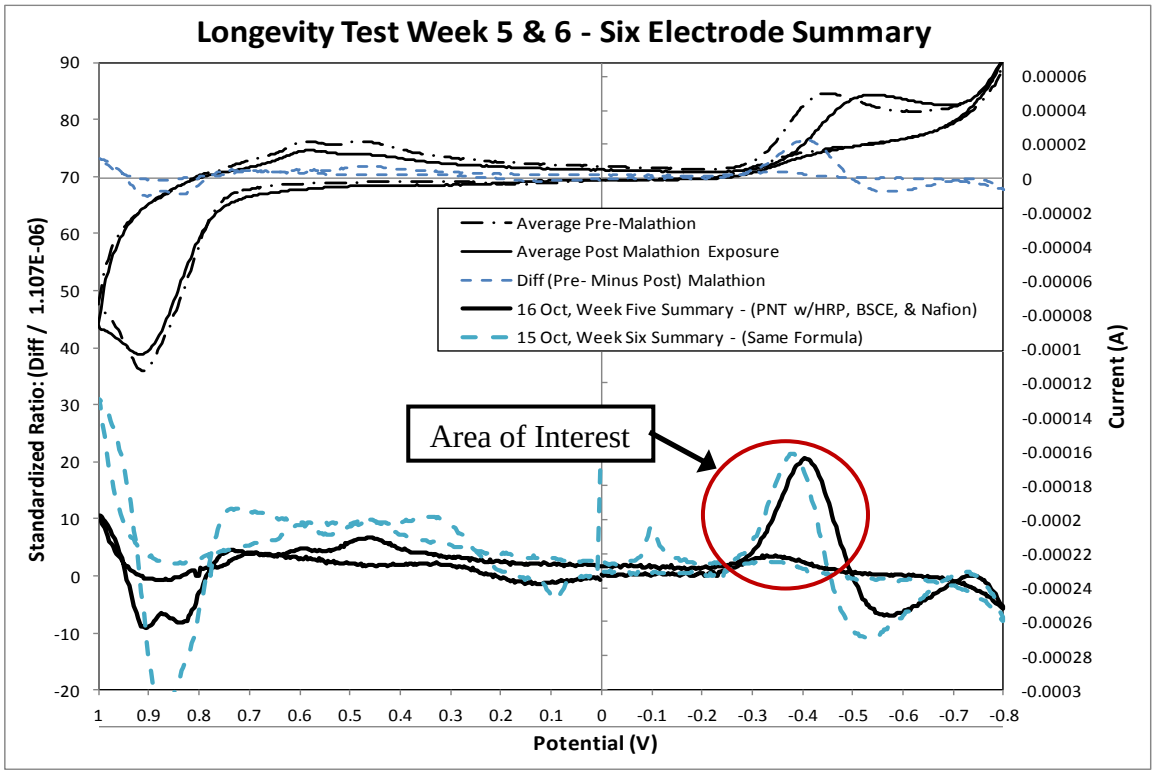

Figure C- 4: Longevity Test Week Five and Six

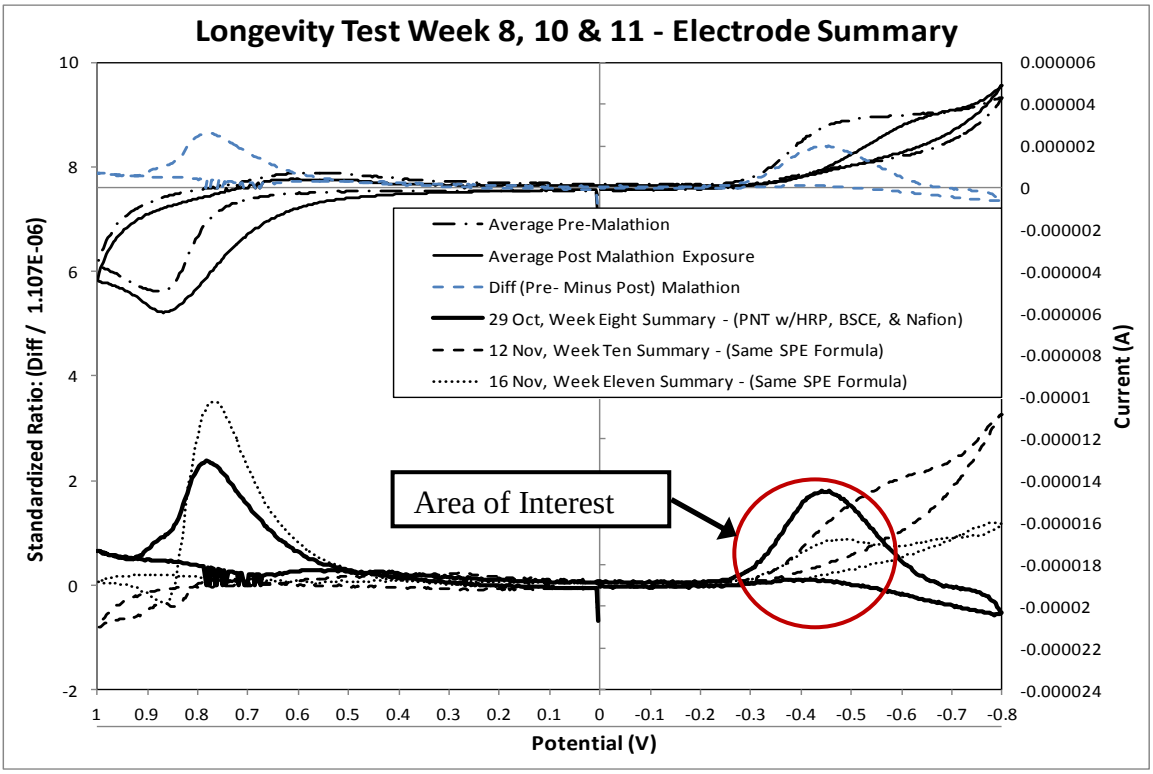

Figure C- 5: Longevity Test Week Eight, Ten and Twelve

Table C-3: Longevity Exposure Experiment Week 5 and 6

Electrode #	Week 5 – 16 Oct 13		Week 6 - 15 Oct 13	
	Potential (V)	**Inhibition (%)**	**Potential (V)**	**Inhibition (%)**
1	-0.34 to -0.35	54.78	-0.32 to -0.34	65.34
2	-0.36 to -0.37	53.95	-0.34 to -0.35	65.04
3	-0.33 to -0.39	58.47	-0.34 to -0.36	61.54
4	-0.34 to -0.37	66.67	-0.33 to -0.35	64.99
5	-0.37 to -0.38	61.79	-0.35 to -0.37	59.25
6	-0.37 to -0.38	60.90	-0.35 to -0.36	38.63*
Result	**-0.35 to -0.37**	**58.98**	**-0.34 to -0.35**	**58.35**
Average		**59.43**		**63.23**
Std Dev		**4.75**		**2.72**

Note: Analysis of CV curve electrode data indicates the biosensor surface may have been abraded during the test protocol series resulting in an erroneous inhibition result.

Inhibition is related to the amount of malathion concentration and the duration of exposure. Measured inhibition is also influenced by BChE "recovery:" a delay in the amount of time between exposure and the follow up test results in an inability to fully capture the inhibition. The BChE/BSCh chemical reaction is *reversible;* when BChE is no longer exposed to malathion, inhibition moves toward the non-exposure, equilibrium state.

Table C-4: Longevity Exposure Experiment Week 8, 10 and 12

Electrode #	Week 8 – 29 Oct 13		Week 10/11 – 12/16 Nov 13[1]	
	Potential (V)	**Inhibition (%)**	**Potential (V)**	**Inhibition (%)**
1	-0.33 to -0.34	76.29	-0.36 to -0.37	39.04
2	-0.34 to -0.35	82.50	-0.35 to -0.35	55.30
3	-0.33 to -0.35	75.01	-0.34 to -0.35	33.82
4	-0.35 to -0.37	82.48	-0.37 to -0.38	3.98
5	-0.34 to -0.37	72.18	-0.35 to -0.36	39.17
6	-0.36 to -0.38	72.72	-0.37 to -0.38	51.06
Result	**-0.34 to -0.36**	**76.61**	**-0.32 to -0.35**	**31.24**
Average		**76.86**		**31.40**
Std Dev		**4.60**		**8.41**

Note: After analysis of longevity week 8 results, it was determined that electrode degradation would likely not provide usable results if the longevity test was carried to week 12. Electrodes 1, 2, and 4 were tested on week 10. Electrodes 4, 5, and 6 were measured on week 11.

Additional Longevity Experiments - 26 Oct 13 through 16 Nov 13

The following data incorporates a second data set of longevity experiments that was collected. As is noted in the comment section of the tables, the two data sets were combined using statistical pooled average and pooled standard deviation calculations. The longevity test results were consistent.

Table C-5: Longevity Exposure Experiment Week 1 and 2

Electrode #	**Week 1 – 26 Oct 13**		**Week 2 - 2 Nov13**	
	Potential (V)	**Inhibition (%)**	**Potential (V)**	**Inhibition (%)**
1	-0.33 to -0.35	69.67	-0.32 to -0.35	63.27
2	-0.32 to -0.35	64.66	-0.37 to -0.38	32.21
3	-0.32 to -0.33	48.86	-0.37 to -0.38	56.29
4	-0.33 to -0.36	51.23	-0.34 to -0.35	60.24
5	-0.33 to -0.36	59.16	-0.34 to -0.35	63.25
6	-0.32 to -0.35	-[3]	-0.36 to -0.37	53.03
Result	**-0.32 to -0.36**	**58.02**	**-0.34 to -0.36**	**53.79**
Average[1] **Std Dev**	**56.63** **8.90**	**58.72** **8.78**	**53.63** **6.78**	**54.72** **11.73**
Pooled Average[1]	**57.68**		**54.17**	
Pooled Std Dev[2]	**8.84**		**9.58**	

Note 1: Pooled Average is the average of all samples taken in each set divided by the number of samples.

Note 2: Pooled Standard Deviation = $\sqrt{(S_p^2)} = \sqrt{\{[(n_1-1)s_1^2+(n_2-1)s_2^2+ \ldots(n_k-1)s_k^2] / [n_{1+} n_{2+} \ldots n_k - k)]\}}$

Note 3: CV Curve was non-consistent and unusable.

Table C-6: Longevity Exposure Experiment Week 3 and 4

Electrode #	Week 3 – 9 Nov 13		Week 4 - 16 Nov 13	
	Potential (V)	**Inhibition (%)**	**Potential (V)**	**Inhibition (%)**
1	-0.32 to -0.35	66.15	-0.32 to -0.35	47.6
2	-0.32 to -0.35	61.70	-0.32 to -0.35	43.16
3	-0.32 to -0.35	-[3]	-0.32 to -0.35	37.59
4	-0.32 to -0.35	50.89	-0.32 to -0.35	23.59
5	-0.32 to -0.35	55.43	-0.32 to -0.35	58.23
6	-0.32 to -0.35	56.79	-0.32 to -0.35	57.29
Result	**-0.32 to -0.35**	**58.19**	**-0.32 to -0.35**	**44.41**
Average	**68.72**	**58.19**	**49.74**	**44.58**
Std Dev	**6.48**	**5.89**	**6.54**	**13.03**
Pooled Average[1]	**63.93**		**47.16**	
Pooled Std Dev[2]	**6.22**		**10.31**	

Note 1: Pooled Average is the average of all samples taken in each set divided by the number of samples.

Note 2: Pooled Standard Deviation = $\sqrt{}$ (S_p2) = $\sqrt{}$ {$[(n_1-1)s_1^2+(n_2-1)s_2^2+ \ldots(n_k-1)s_k^2] / [n_1+ n_2+ \ldots n_k - k)]$}

Note 3: CV Curve was non-consistent and unusable.

Appendix D. Chemical/Materials Ordering List

Table D-1: Material List, Amount, and Manufacturer Source

Chemical Type HAZ Code	CAS Number Product #	Manufacturer	Additional Info
ASCh-Chloride HAZ B (213089)	60-31-1 A6635	Sigma Aldrich St. Louis, MO	>99% TLC 25G $40.60 1001053000
AChE-type V-S from electric eel HAZ B (213069)	9000-81-1 C2888	Sigma Aldrich Milwaukee, WI	500UN $78.00 1001165276
Horseradish Peroxidase (HRP) HAZ B (213103)	9003-99-0 P8250	Sigma Aldrich Milwaukee, WI	5KU $43.48
Butyrylthiocholine (BSCh) HAZ B (216566)	22026-63-7 B3128	Sigma Aldrich St. Louis, MO	1G $102.00
Butyrylcholinesterase (BChE) HAZ A (216568)	9001-08-5 C1057 C4290	Sigma Aldrich St. Louis, MO	1KU $244.56 1KU $210.00
Cellulose acetate HAZ A (216567)	9004-35-7 180955	Sigma Aldrich St. Louis, MO	25G $37.60 500G $74.50
Malathion >95% HAZ C (214175)	121-75-5 36143 91481	Sigma Aldrich Milwaukee, WI	100MG $33.90 50MG $77.10
H-Phe-Phe-OH (DI-L-Phenylalanine) HAZ C (213072)	150-30-1 147966	Sigma Aldrich St. Louis, MO	101069369 25G $30.80
1,1,1,3,3,3-hexafluoro-2-propanol (99.8% purity) HAZ B (213090)	920-66-1 105228	Sigma Aldrich Milwaukee, WI	>99% 25G $85.74
Nafion© 117 solution (approx. 5%)	31175-20-9 309389	Sigma Aldrich Allentown, PA	25G $258.50
Deionized water	-	AFIT Lab Dayton, OH	
Ammonium acetate HAZ A (203063)	631-61-8 A1542	Sigma Aldrich Allentown, PA	250G $31.00
Potassium phosphate dibasic HAZ B (153837)	7758-11-4 P3786	Sigma Aldrich Allentown, PA	1KG $105.09
Gold SPEs model DRP-250 (a 4-mm-diameter gold)	N/A	Metrohm USA Riverview, FL	Mike Kubicsko 516-644-0354
Electrode-Potentiostat interface cable	N/A	Metrohm USA Riverview, FL	
Jacketed Compact Voltammeter Cell	N/A	Pine Research Instrumentation Durham, NC	

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14. ABSTRACT

Previous work to develop biosensors that can be used to detect organophosphorus compounds (OPCs) has successfully demonstrated the potential application of enzymes encapsulated in peptide-nanotubes (PNTs) enhanced with horseradish peroxidase (HRP) to detect the presence of OPCs in the aqueous and gas phases. In this research, a standardized test method that was applied to evaluate a biosensor fabricated with a single-use electrode, was refined to accommodate a reusable screen printed electrode. Also in this study, butyrylcholinesterase (BChE) enzyme was used in lieu of the acetylcholinesterase (AChE) enzyme applied in earlier studies in an effort to enhance biosensor performance.

Biosensor operation is based on the principle that butyrylthiocholine (BSCh), in the presence of the enzyme BChE, will produce a measurable electrochemical signal during chemical reaction; a signal that is inhibited in the presence of an OPC. For this research, cyclic voltammograms (CVs) were used to measure the inhibition in current due to the presence of a model OPC, malathion. The response of a BChE-based biosensor was shown to be inhibited by gas phase malathion concentrations less than 25 ppbv, with the extent of inhibition linearly proportional to the malathion concentration above 6 ppbv. Additionally, this study demonstrated that a BChE-based biosensor stored at room temperature can be used as long as 42 days after fabrication.

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